正常人尿液蛋白质组非限制修饰鉴定与比较：多位点氧化修饰是不同年龄段人群的尿蛋白修饰特点

刘永涛，潘宣圳，华元瑞，王云龙，
高友鹤*
生物化学与分子生物学系，基因工程药物及生物技术北京市重点实验室，生命科学学院，北京师范大学，北京 100875
*联系人：E-mail：**gaoyouhe@bnu.edu.cn**

摘要 健康人尿液中的蛋白质含量虽较低，但种类却十分丰富，尿蛋白质组所蕴含信息能够展现机体生理与病理早期阶段时的细微差别。蛋白质的化学修饰是指其氨基酸残基或其链末端上参与的共价基团反应，进而使其分子的结构、执行的调控和信息传递的功能得到改变，因此，对尿液蛋白质化学修饰水平的比较与研究同样有重要意义。本研究拟以不同年龄段健康人群的尿液（儿童 22 例，年轻人 10 例，老年人 6 例）为研究对象，通过高分辨串联质谱及非标记定量的蛋白质组学分析方法，结合非限制性修饰鉴定算法整体比较三种类型样品之间的蛋白质化学修饰差异水平。结果表明，涉及多种氨基酸残基的氧化修饰是老年人与青少年尿液蛋白质样品之间的主要差异，且修饰影响了众多蛋白质的生物过程。本研究首次利用非限制性修饰鉴定算法研究了不同年龄段人群的尿液在整体蛋白质组修饰上的差异，并指出了氧化修饰是区分青少年和老年人尿液蛋白质的主要修饰类型。

关键词 氧化修饰；整体化学修饰；非限制性修饰鉴定算法；尿液；蛋白质组

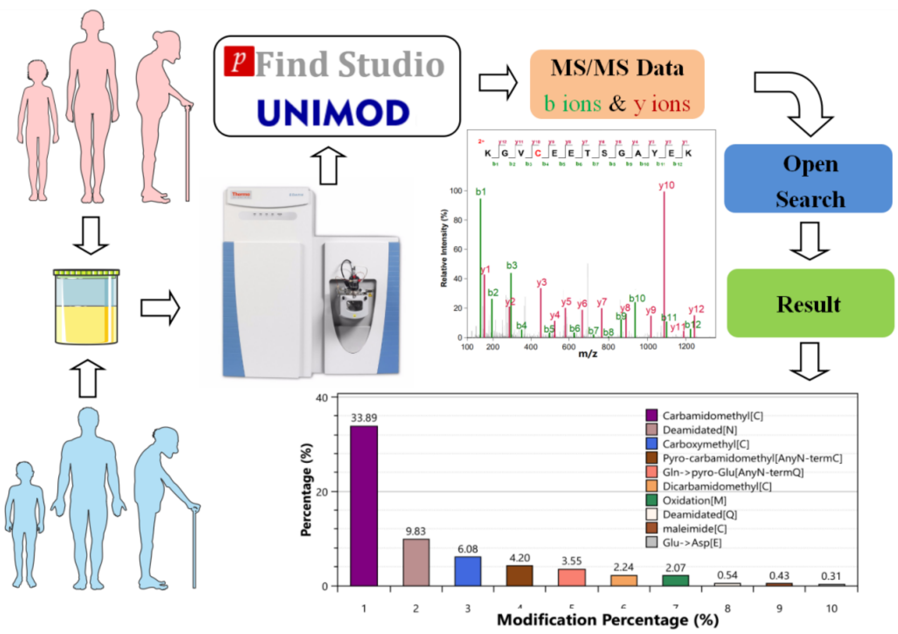
引言

生命体中的生物过程能同时且相互协调的运作，离不开蛋白质所参与的合成、催化与调节等众多生化反应。蛋白质是一类结构复杂的生物大分子，高级结构的差异决定了其各自所具有生物化学活性的不同，蛋白质之间通过相互组合形成更高级的网络来执行特定的功能 [1]。蛋白质的化学修饰是指其氨基酸残基或其链末端上参与的共价基团反应，化学修饰通常会改变蛋白质的高级结构，其中少数的化学结构改变并不影响蛋白质的生物学活性，这些被称为蛋白质非必需部分的修饰，但绝大多数情况下分子结构的改变，会显著改变蛋白质物理及化学性质，改变蛋白质的构象，会使蛋白质的活性发生变化，进而使其执行的功能得到改变 [2]。所以，即使在蛋白质含量水平没有变化，但如果其化学修饰水平发生了某些微小的变化，也会使蛋白质的功能显著变化，这意味着蛋白质的化学修饰在另一个维度上丰富了蛋白质行使的不同功能及调节相关蛋白质的功能。蛋白质的化学修饰对蛋白质功能的影响主要表现在以下三个方面：1. 即使蛋白质发生了一种修饰也会对其功能造成影响。2.同一种蛋白质，不同氨基酸发生了同种或不同种修饰对其功能造成的影响也不同。3.同一种蛋白质可能发生多种类型的化学修饰，进而使其参与的生物过程更加多变与复杂 [3]。与蛋白质相关的化学修饰主要有以下几种类型：1.蛋白质的翻译后修饰(Post-translational modification, PTM)[4]，是指蛋白质在翻译后进行的化学修饰，翻译后修饰蛋白前体往往是没有生物活性的，通常需要经过特定修饰酶的翻译后修饰加工，才能成为具有功能的成熟蛋白质，行使其具体生物功能 [5,6]。2.蛋白质的化学衍生修饰(Chemical derived modification)是指在蛋白质侧链中引入新的基团或去除原有基团的一类修饰，通常包含自发性非酶促修饰，也有交联剂与人工试剂引入的修饰等。3.氨基酸的替换(Amino acid substitution)，是指蛋白质侧链中原有氨基酸被其他种类氨基酸所替换所引起的蛋白质性质与功能的改变。这些都是会显著影响蛋白质功能的修饰类型。

质谱分析技术不但能够实现大规模组学数据的采集与深度挖掘，也能够对特定蛋白质的靶向修饰实现准确测定。随着仪器科学的不断发展，超高分辨率及串联质谱技术(LC-MS/MS)为蛋白质组学及其修饰的研究提供了更多丰富与精准的信息与数据，这也为准确鉴定蛋白质链中化学修饰位点提供了有力的帮助。通

常进行蛋白质组数据分析时需进行物种蛋白质组数据库搜索比对，在使用搜索引擎进行数据库检索时，通常需要人为设定已知的蛋白质化学修饰类型，这种类型的搜索方式称为限制性搜索，而对于样品中蛋白质修饰类型未知，期望寻找新的修饰类型时则显得难以实现[7]。因此，全面、非限制修饰搜索对于了解样品蛋白质组所包含的全部化学修饰信息有着至关重要的地位，Open-pFind 算法是一种开放式序列库搜索算法，其将 UniMod 数据库的信息整合，通过开放式搜索的方式对采集到的质谱数据进行分析处理，可得到蛋白质组的整体化学修饰信息[8-10]。

健康人尿液中虽然蛋白质总量较低，但种类却异常丰富。已知正常人尿液中可以鉴定到 6000 余种不同的蛋白质。正常情况下，肾小球可筛过一部分蛋白质，其中 98%在肾小管被重吸收回体内，剩余 2%的蛋白质与肾小管及其他尿路上皮细胞分泌的少量黏蛋白一起排出。尿液产自肾脏，并在膀胱中储存了几个小时，因此，尿液中蛋白质信息可直观地反映泌尿系统的健康状况。但尿液中大多数蛋白质是通过肾小球过滤血浆后汇集在一起的，理论上它们都来源于血液，在经过肾脏的过滤与重吸收依旧能够保留下来，足够说明剩余的 2%蛋白质也不可能是被健康的肾脏随机、非选择性地“漏出”。尿液蛋白质组学另一个重要的研究方向便是其化学修饰领域，全面地比较尿液蛋白质组化学修饰水平的变化也将会为研究机体生理方面的变化提供更加丰富的信息[6]。目前，被收录在 UniMod[11]、PSI-MOD、RESID 数据库中的蛋白质化学修饰种类已达 1500 余种。血浆蛋白质组能够反映出年龄与衰老之间的细微状况[12]，作为另一种同样富含蛋白质信息的体液—尿液，通过对其研究发现尿蛋白质组也具有反映出机体衰老信息能力[13]，同时在我们先前对新出生的胎鼠尿液蛋白质组研究，同样发现出蛋白表达的显著差异。甚至是饥饿这种常见的生理过程都可以在尿蛋白质组中找到线索[14]。基于此，本研究基于不同年龄段人群尿液蛋白质组中化学修饰的重要性，通过高分辨串联质谱结合非限定性修饰搜索(Open-pFind)，拟比较三组不同年龄段人群尿液蛋白质组整体化学修饰水平的差异。



1. 材料与方法

1.1 样本与数据来源

22 例健康儿童尿液蛋白质组数据来源于一项关于膀胱输尿管反流复发性尿路感染患者的研究(Raw 数据下载地址：http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD010469）。6 例健康老年人尿液蛋白质组数据来源于一项尿路中性粒细胞与相关性炎症的研究（Raw 数据下载地址 http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD004713）,以上下载数据均为健康组对照样本。

10 例健康青年人尿液样本从实验室的健康志愿者中收集，对志愿者的饮食、药物和其他因素没有任何限制或要求。本实验已向志愿者提供研究的详细情况，内容包括研究目的、方法及过程，同时对志愿者的个人资料严格保密。样本信息详见表 1。

表 1 下载数据及收集的样本信息与统计结果

Table 1 Download data,collect sample information and statistics

	健康尿液样本（n=38）		
	儿童（n=22）	青年人（n=10）	老年人（n=6）
性别			
男	13	5	2
女	9	5	4
年龄			
平均值	5.7±3	26.4±2	76.5±5
年龄范围	1~22	22~29	70~81

1.2 蛋白质样品制备和胰蛋白酶酶解

青年人的尿液样本使用 20 mmol / L 的二硫苏糖醇(DTT)在 37 ℃下与样品反应 1 h，使蛋白质结构中的二硫键变性，随后添加 55 mmol / L 的碘乙酰胺(IAA)并避光反应 30 min 以使二硫键结合位点烷基化。在-20℃下，用三倍体积的预冷丙酮沉淀上清液 2 h，然后在 4 ℃和 12,000 ×g 下离心 30 min，获得蛋白沉淀。随后将沉淀物重悬于适量蛋白溶解液（8 mol/L 尿素，2 mol/L 硫脲，25 mmol/L DTT 和 50 mmol/L Tris）中。使用 Bradford 分析法测量蛋白质提取液浓度。通过使用滤器辅助样品制备（FASP）的方法[15]，每个样品按 50：1 的比例使用胰蛋白酶（Trypsin Gold, Mass Spec Grade, Promega, Fitchburg, WI, USA）酶解 100 μg 的蛋白质。37 ℃酶解 14 h 后，向溶液中加入 10%甲酸溶液终止酶解，经 10 KDa 超滤管离心后获得多肽溶液，使用 BCA 法测定多肽的浓度，并通过真空离心浓缩仪（Thermo Fisher, USA）进行干燥，干燥后的多肽密封至于-80 ℃保存。表 2 展示了文献中其他两类人群尿液样本处理方法，并与本方法进行了比较。

表 2 几组尿液样本处理方法的比较

Table2 Comparison of treatment methods of urine samples

	儿童尿液	青年人尿液	老年人尿液
样本预处理	4500 × g，离心 20min；	2500 × g 18 ℃，离心 15min；	2500 × g 10℃，离心 15min；
蛋白质富集与浓缩	10 kDa 滤器离心富集；	预冷丙酮沉淀富集；	10 kDa 滤器离心富集；
还原与烷基化方法	DTT/IAA 法；	DTT/IAA 法；	DTT/IAA 法；
蛋白质的酶解	Solid-phase Reversible Sample-preparation (SRS)法；	滤器辅助样品制备 (FASP)法；	FASP 法；
蛋白酶类型	胰蛋白酶；	胰蛋白酶；	胰蛋白酶；
多肽脱盐	文中未提及；	C18 SPE；	Stage tip；

1.3 液相色谱-串联质谱（LC-MS / MS）分析和数据库搜索

健康青年人的尿液样本分析前，需将干燥的多肽样品溶于 0.1%甲酸，控制终浓度在 0.1 μg/μL，每个样品按 1 μg 多肽质量进行分析：Thermo EASY-nLC1200 色谱系统加载至预柱与分析柱上。通过 Thermo Orbitrap Fusion Lumos 质谱系统(Thermo Fisher Scientific, Bremen, Germany) 采集蛋白质组数据。液相色谱

分析方法：预柱：75 μm×2 cm, nanoViper C18, 2 μm, 100Å；分析柱：50 μm×15 cm, nanoViper C18, 2 μm, 100 Å；进样体积：10 μL，流速：250 nL/min。流动相配置如下，A 相：100%质谱级水(Fisher Scientific,Spain)/1‰甲酸(Fisher Scientific)，B 相：80%乙腈(Fisher Scientific,USA)/20%水/1‰甲酸，120 min 梯度洗脱：0 min，3% B 相； 0 min-3 min，8% B 相；3 min- 93 min，22% B 相；93 min- 113 min，35% B 相；113 min- 120 min，90% B 相； 质谱分析方法，离子源：nanoESI，喷雾电压：2.0 kV，毛细管温度：320 ℃，S-lens RF Level: 30，分辨率设置：一级（Orbitrap）120,000 @m/z 200，二级 30,000（Orbitrap）@m/z 200，母离子扫描范围：m/z 350-1350；子离子扫描范围：从 m/z 110 开始，MS1 AGC: 4e5，电荷范围：2-7，离子注入时间：50 ms，MS2 AGC: 1e5，离子注入时间：50ms，离子筛选窗口：2.0 m/z，碎裂模式：高能碰撞解离（HCD），能量：NCE 32，Data-dependent MS/MS：Top 20，动态排除时间：15 s，内部校准质量数：445.12003。表 3 展示了文献报道中其他两种人群尿液样本数据采集方法，并与本方法进行了比较。

表 3 几组尿液蛋白质组样本仪器分析方法的比较

Table 3 Comparison of instrument analysis methods for urine proteome samples

	儿童组尿液样本	青年组尿液样本	老年组尿液样本
超高效液相色谱仪	nanoflow UPLC system (Eksigent, Dublin,CA)	Easy-nanoLC 1200 (Thermo Scientific) ;	Ultimate 3000 nano LC (Thermo Scientific)
高分辨质谱仪	Q Exactive (Thermo Scientific);	Orbitrap Fusion Lumos (Thermo Scientific);	Q Exactive (Thermo Scientific);
捕集柱	Magic C18, 5 μm, 100 Å (Michrom BioResources /Bruker, Billrica, MA) ;	C18 nanoViper, 75 μm×2 cm, 2 μm, 100 Å (Thermo Scientific);	C18 PepMap100, 300 μm × 5 mm, 5 μm, 100 Å (Thermo Scientific);
分析柱	PicoTips (15 cm x 100 μm ID; New Objective, Woburn, MA);	C18 nanoViper, 50 μm×15 cm, 2 μm, 100 Å (Thermo Scientific);	75 μm×10 cm, 5 μm BetaBasic C18, 150 Å, (New Objective, MA);
流动相 A	0.1%甲酸-水	0.1%甲酸-水	0.1%甲酸-水
流动相 B	100%乙腈-0.1%甲酸	80%乙腈/水-0.1%甲酸	100%乙腈-0.1%甲酸
流速	文中未提及	250 nL / min	300 nL / min
梯度洗脱时间	127 min	120 min	130 min
梯度洗脱程序	0 min, 5% B 相, 0~120 min，30% B 相, 120~127 min，95% B 相；	0 min, 3% B 相, 0~3 min, 8% B 相, 3~93 min, 22% B 相, 93~113 min, 35% B 相, 113~120 min, 90% B 相;	0 min, 0% B 相, 0~110 min，35% B 相, 110~125 min, 80% B 相， 125~130 min, 80% B 相;
喷雾电压	文中未提及	1.9 kV;	2.1 kV;
MS1 分辨率	文中未提及	120,000 (m/z 350~1350);	70,000 (m/z 250~1800);
MS2 分辨率	文中未提及	30,000;	17,500;
碎裂模式	文中未提及	HCD 32%	HCD 27%

使用 pFind Studio 软件（3.1.6 版，中国科学院计算技术研究所）对 LC-MS / MS 数据进行无标记的定量分析。目标检索数据库来自 Uniprot 下载的 Homo Sapiens 数据库（更新至 2020 年 2 月），检索时，仪器类型为 HCD-FTMS，酶全特异性，为胰蛋白酶，最多有 2 个漏切位点。选择“开放式搜索（open-search）”。筛选条件：在肽水平上的 FDR 小于 1%，在蛋白水平上的 Q 值小于 1%。数据同时使用正向和反向数据库检索策略来分析数据。

1.4 统计与分析

对于 pFind 提供的数据进行描述性统计计算与分析，例如四分位数范围的中位数（对于偏斜分布的数据）和比例（百分比）。 通过 t 检验，方差分析，Mann-Whitney U 检验和 Kruskal-Wallis 检验评估儿童组、青年组和老年组组之间的统计比较。使用 GraphPad Prism v7.04 执行统计分析。 p 值< 0.05 被认为有显著性。

2. 结果

2.1 通过使用 bottom-up 的蛋白质组学技术对总蛋白进行鉴定

依靠非标记定量蛋白质组的方法，通过 LC-MS/MS 分析获得了 38 个样本的实验结果。在基于 open-pFind 软件检索数据（.raw）之后，可以在 pBuild 中浏览并导出分析结果。将样品结果进行整理和统计，样品中蛋白质和多肽的鉴定结果如表 4 所示。

表 4 尿液样品中蛋白质和多肽的鉴定结果

Table 4 The results of proteins and peptides identification in urine samples

	Spectra	**Scans**	**Peptides**	**Sequences**	**Protein Groups**
儿童组	29239 ± 5406	24763 ± 3833	13003 ± 2892	10002 ± 2252	2787 ± 898
青年组	57001 ± 4820	42944 ± 2670	18024 ± 1562	12743 ± 1376	3447 ± 811
老年组	27007 ± 6575	23651 ± 5065	7906 ± 1899	5445 ± 1402	1593 ± 441

2.2 整体化学修饰信息与差异修饰统计

在 38 个样本中共鉴定到 485 种不同化学修饰类型，其中儿童组共鉴定出 301 种化学修饰类型，青年组共鉴定到 329 种化学修饰类型，老年组共鉴定到 106 种化学修饰类型。**图 1** 展示了三种类型样本间修饰类型的交互关系图(韦恩图)，交互部分具体内容参见**附表 1**。

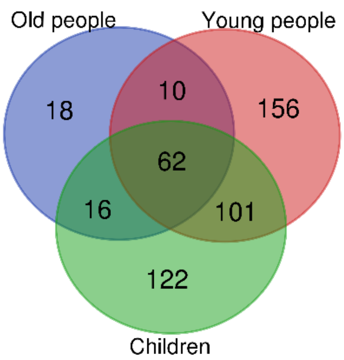


图 1 样本间修饰类型的交互关系

Fig1 The Venn of different modification types between samples

对整体修饰和共有的 62 种修饰进行非监督聚类分析，在共有修饰的热图中发现老年组一个样本和青年组两个样本被分在儿童组分支下，在这两个热图中均发现同一个老年组的样本比较异常，被分在儿童和青年组中，除此之外，两图中老年人组和青年以及儿童组可以区分开，详见**图 2**、**图 3**。共有 40 种修饰存在统计差异（p 值小于 0.05），通过计算与统计得到每种修饰在不同组间的变化情况，详见**表 5**。每种差异修饰不同组间进行了比较，具体情况见**图 4** 从 40 种差异修饰中选取组间显著差异的修饰如下：脯氨酸、色氨酸、酪氨酸、半胱氨酸、甲硫氨酸的氧化修饰，半胱氨酸变成脱氢丙氨酸(Cys->Dha [C])，环氧丙酰胺的 N 端修饰(glycidamide [AnyN-term])，丝氨酸的磷酸化(Phospho [S])，天冬氨酸和谷氨酸上的两个质子被钙离子取代(Cation_Ca [E] 、Cation_Ca [D])，蛋白质 N 端的琥珀酰化(Succinyl [AnyN-term])，蛋白质 N 端的氨甲酰化(Carbamyl [AnyN-term])，缬氨酸被苏氨酸取代(Val->Thr [V])，谷氨酸被甲硫氨酸取代(Glu->Met [E])，

谷氨酰胺取代谷氨酸(Glu->Gln [E])，谷氨酰胺脱酰氨化修饰 Deamidated [N] ，半胱氨酸的脱氢 DiDehydro [C]等。因为氧化修饰差异显著，且和年龄的相关更加明显，我们查找到 485 种修饰中所有的氧化修饰，包括脯氨酸、色氨酸、酪氨酸、半胱氨酸、甲硫氨酸的氧化修饰以及其他不显著非差异的氧化修饰，进行聚类分析，发现可以很好的区分开三组不同年龄段，详见**图 5**。

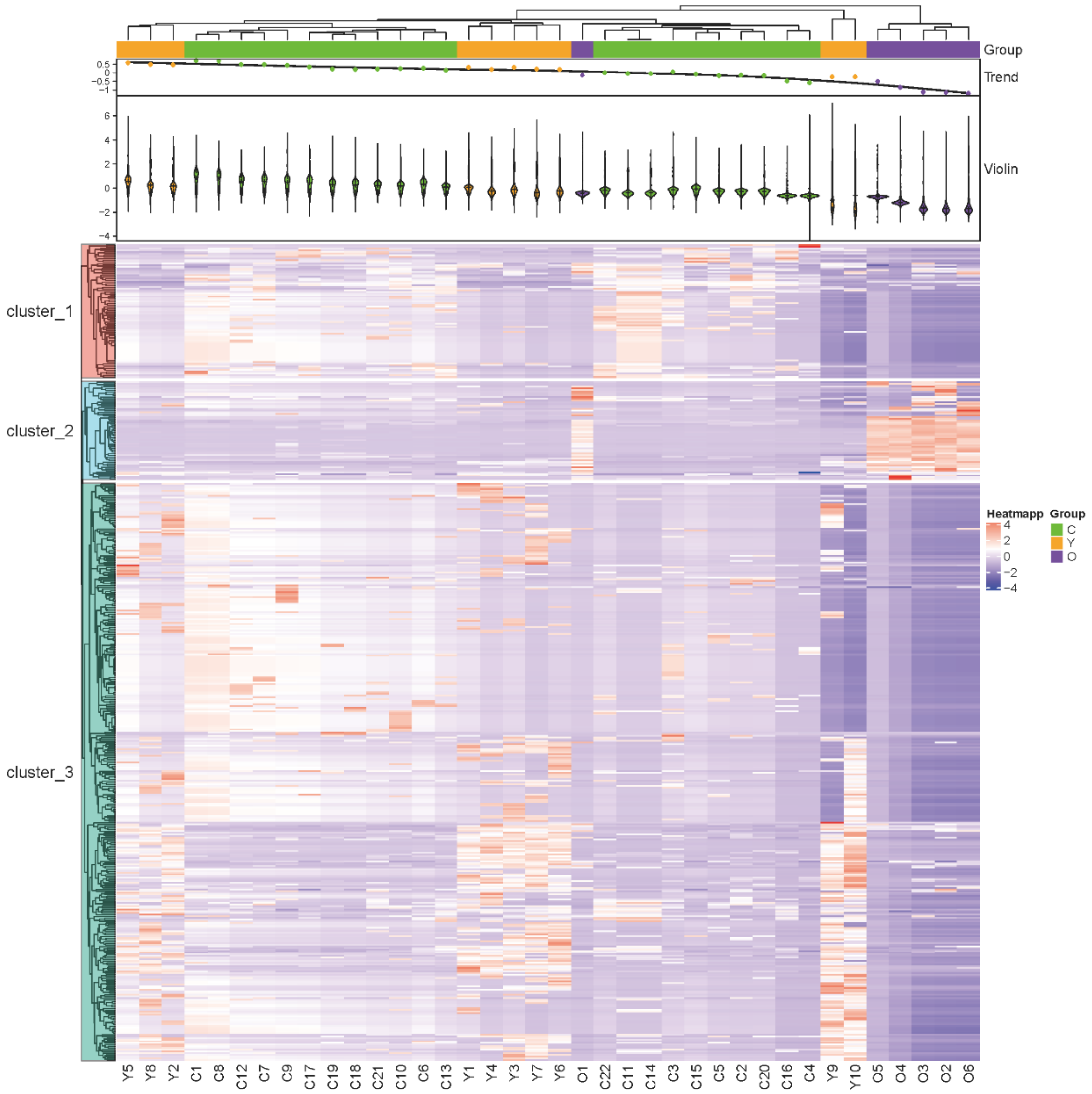


图 2 三组样本总体修饰的非监督聚类分析结果。绿色为儿童组样本，橙色为青年组样，紫色为老年组样本；

Fig 2 Cluster analysis results of the total modifications for three groups samples. Green is the sample of children group, and orange is the sample of young people group, purple is the sample old people group;

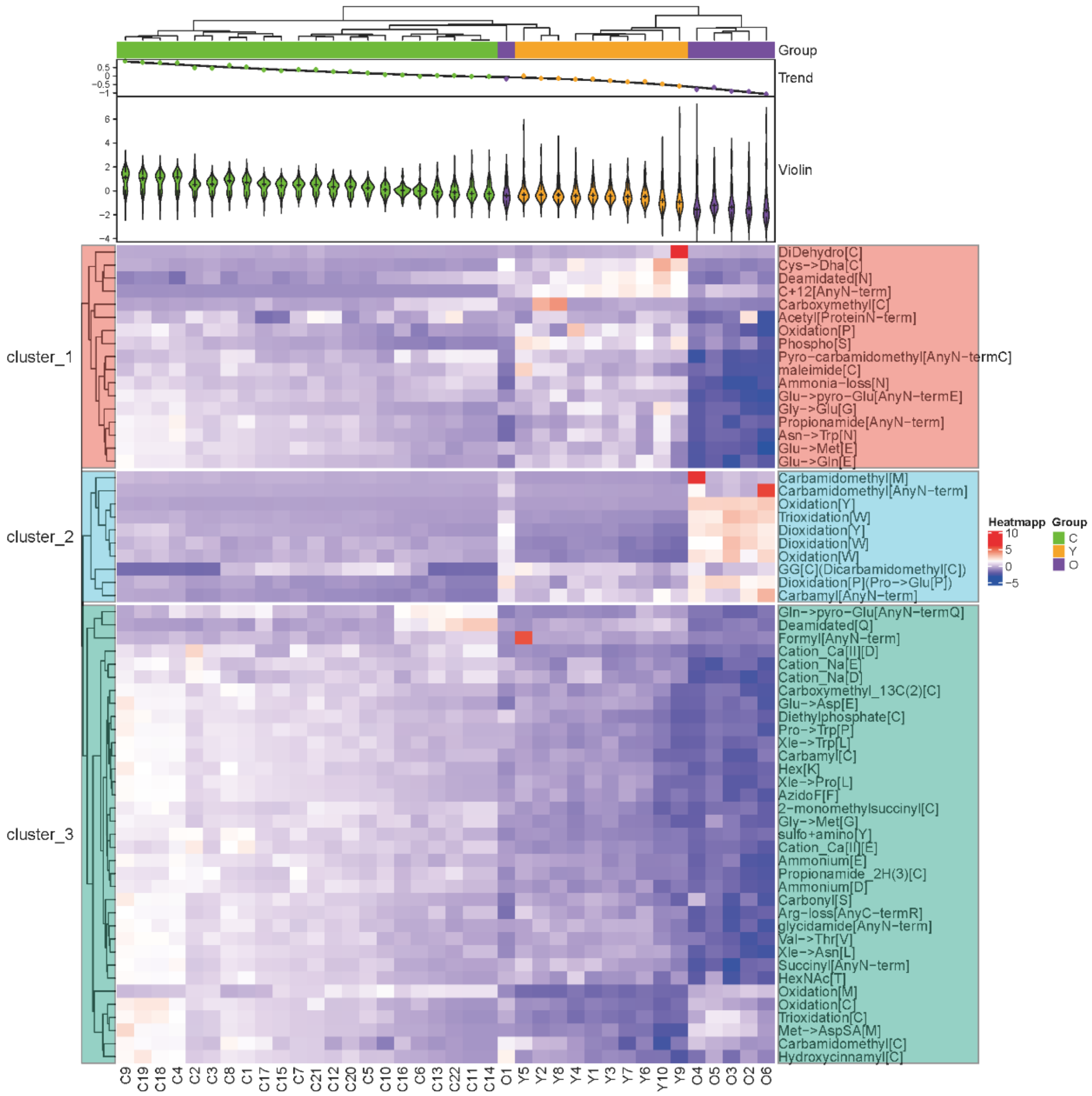


图 3 三组样本间共有修饰的聚类分析结果。绿色为儿童组样本，橙色为青年组样，紫色为老年组样本；

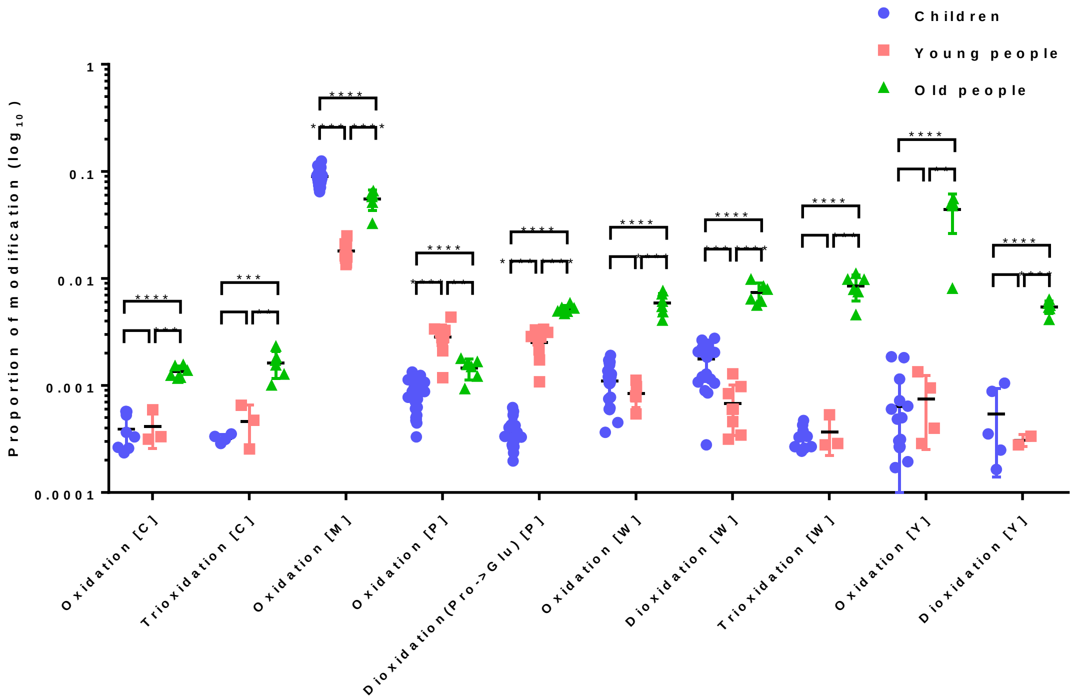
Fig 3 Cluster analysis results of the common modifications among the three groups of samples. Green is the sample of children group, and orange is the sample of young people group, purple is the sample old people group;

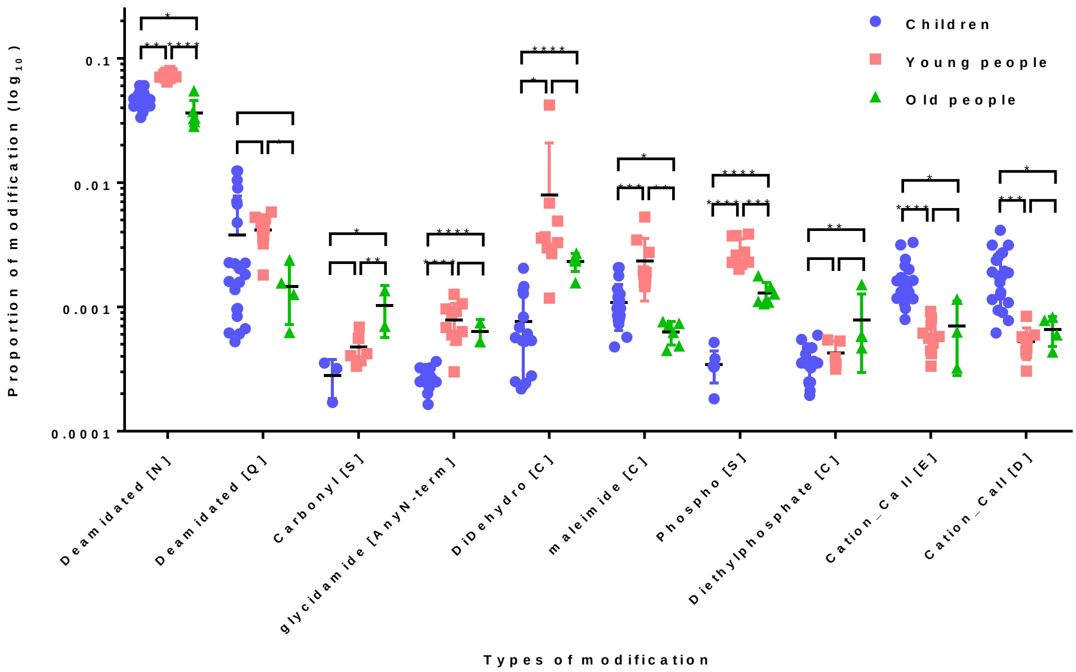
表 5 不同组间差异化学修饰情况与统计

Table 5 Differences and statistics of chemical modifications between different groups

修饰名称	修饰类型	老年组/儿童组		老年组/青年组		儿童组/青年组	
		变化倍数	*p* 值	变化倍数	*p* 值	变化倍数	*p* 值
Oxidation [C]	翻译后修饰	3.46 ↑	9.73E-08	3.26 ↑	1.06E-04	1.06 ↓	>0.05
Trioxidation [C]	化学衍生	5.04 ↑	5.52E-04	3.52 ↑	4.72E-03	1.43 ↓	>0.05
Oxidation [M]	人工引入	1.61 ↓	3.38E-05	3.05 ↑	2.42E-07	4.93 ↑	6.29E-15
Oxidation [P]	翻译后修饰	1.79 ↑	5.88E-05	1.96 ↓	1.65E-03	3.57 ↓	1.97E-11
Dioxidation(Pro-> Glu) [P]	翻译后修饰	14.11 ↑	2.99E-21	2.06 ↑	1.21E-06	6.67 ↓	2.32E-11
Oxidation [W]	人工引入	5.36 ↑	1.61E-13	6.99 ↑	1.93E-05	1.30 ↑	>0.05
Dioxidation [W]	化学衍生	4.17 ↑	6.64E-13	10.94 ↑	7.10E-08	2.62 ↑	1.76E-04
Trioxidation [W]	化学衍生	1.76 ↑	8.35E-08	23.11 ↑	6.53E-04	1.11 ↓	>0.05

Oxidation [Y]	翻译后修饰	69.11 ↑	6.96E-09	59.04 ↑	1.42E-03	1.18 ↓	>0.05
Dioxidation [Y]	翻译后修饰	10.06 ↑	3.91E-07	17.67 ↑	9.79E-05	1.76 ↑	>0.05
Deamidated [N]	人工引入	1.32 ↓	3.29E-03	2.00 ↓	7.29E-08	1.52 ↓	2.64E-11
Deamidated [Q]	人工引入	2.56 ↓	>0.05	2.86 ↓	1.06E-08	1.10 ↓	>0.05
Carbonyl [S]	化学衍生	3.64 ↑	>0.05	2.15 ↑	1.41E-02	1.69 ↓	4.83E-02
glycidamide [AnyN-term]	化学衍生	2.41 ↑	1.84E-06	1.23 ↓	>0.05	2.94 ↓	4.26E-07
DiDehydro [C]		3.03 ↑	4.16E-06	3.45 ↓	>0.05	10.00 ↓	4.04E-02
maleimide [C]	化学衍生	1.72 ↓	2.01E-03	3.70 ↓	4.48E-03	2.17 ↓	2.04E-04
Phospho [S]	翻译后修饰	3.75 ↑	3.98E-06	2.17 ↓	1.74E-04	8.33 ↓	1.45E-07
Diethylphosphate [C]	化学衍生	2.19 ↑	4.61E-03	1.85 ↑	>0.05	1.19 ↓	>0.05
Cation_Ca[II][E]	人工引入	2.38 ↓	2.31E-02	1.19 ↑	>0.05	2.80 ↑	2.24E-05
Cation_Ca[II][D]	人工引入	2.78 ↓	2.01E-02	1.24 ↑	>0.05	3.48 ↑	2.68E-04
Formyl [AnyN-term]	翻译后修饰	6.67 ↓	2.17E-02	11.11 ↓	>0.05	1.79 ↓	>0.05
2-monomethylsuccinyl [C]	化学衍生	1.04 ↑	>0.05	2.02 ↑	1.07E-02	1.95 ↑	3.03E-02
Succinyl [AnyN-term]	翻译后修饰	3.30 ↑	1.01E-04	1.04 ↓	>0.05	3.45 ↓	5.34E-06
Pyro-carbamidomethyl [AnyN-termC]	化学衍生	2.22 ↓	2.75E-05	2.86 ↓	4.27E-06	1.28 ↓	4.83E-03
Carbamidomethyl [C]	化学衍生	1.25 ↓	1.37E-09	1.27 ↓	6.71E-03	1.01 ↓	>0.05
Carbamidomethyl [M]	化学衍生	6.68 ↑	2.86E-03	19.79 ↑	>0.05	2.96 ↑	3.44E-06
Propionamide [AnyN-term]	化学衍生	1.65 ↑	3.08E-02	1.82 ↓	>0.05	3.03 ↓	7.41E-03
Carbamyl [AnyN-term]	多种	15.65 ↑	2.55E-03	1.31 ↑	>0.05	12.50 ↓	1.32E-05
Acetyl [ProteinN-term]	多种	1.03 ↓	>0.05	1.39 ↓	4.85E-02	1.35 ↓	5.84E-03
Hydroxycinnamyl [C]	翻译后修饰	3.76 ↑	3.66E-02	2.58 ↑	1.21E-02	1.45 ↓	>0.05
Val->Thr [V]	氨基酸替代	1.64 ↑	8.68E-03	1.35 ↓	>0.05	2.22 ↓	2.55E-06
Asn->Asp [N]	氨基酸替代	2.04 ↓	>0.05	5.00 ↓	4.64E-06	2.44 ↓	7.20E-10
Xle->Trp [L]	氨基酸替代	2.21 ↑	1.45E-03	1.54 ↑	>0.05	1.43 ↓	2.71E-02
Gln->pyro-Glu [AnyN-termQ]	人工引入	2.44 ↓	1.63E-07	1.72 ↓	2.64E-06	1.43 ↑	8.52E-05
Glu->pyro-Glu [AnyN-termE]		1.06 ↑	>0.05	2.94 ↓	2.01E-03	3.13 ↓	9.54E-12
Glu->Met [E]	氨基酸替代	2.77 ↑	1.08E-03	1.35 ↓	>0.05	3.70 ↓	5.31E-06
Glu->Gln [E]	氨基酸替代	2.31 ↑	3.14E-04	2.33 ↓	9.41E-04	5.26 ↓	9.51E-09
Gly->Glu [G]	氨基酸替代	2.40 ↑	2.44E-02	1.92 ↓	>0.05	4.55 ↓	1.41E-03
Cys->Dha [C]	化学衍生	2.07 ↑	3.90E-04	2.33 ↓	1.13E-03	4.76 ↓	6.30E-14
C+12 [AnyN-term]		62.20 ↑	2.02E-03	3.57 ↓	1.25E-07	100.0 ↓	1.15E-09


Children
Young people
Old people
Proportion of modification (log10)
1
0.1
0.01
0.001
0.0001
Oxidation [C]
Trioxidation [C]
Oxidation [M]
Oxidation [P]
Dioxidation(Pro->Glu) [P]
Oxidation [W]
Dioxidation [W]
Trioxidation [W]
Oxidation [Y]
Dioxidation [Y]
Types of oxidation modification


Children
Young people
Old people
Proportion of modification (log10)
0.1
0.01
0.001
0.0001
Deamidated [N]
Deamidated [Q]
Carbonyl [S]
glycidamide [AnyN-term]
DiDehydro [C]
maleimide [C]
Phospho [S]
Diethylphosphate [C]
Cation_Ca II [E]
Cation_Ca II [D]
Types of modification

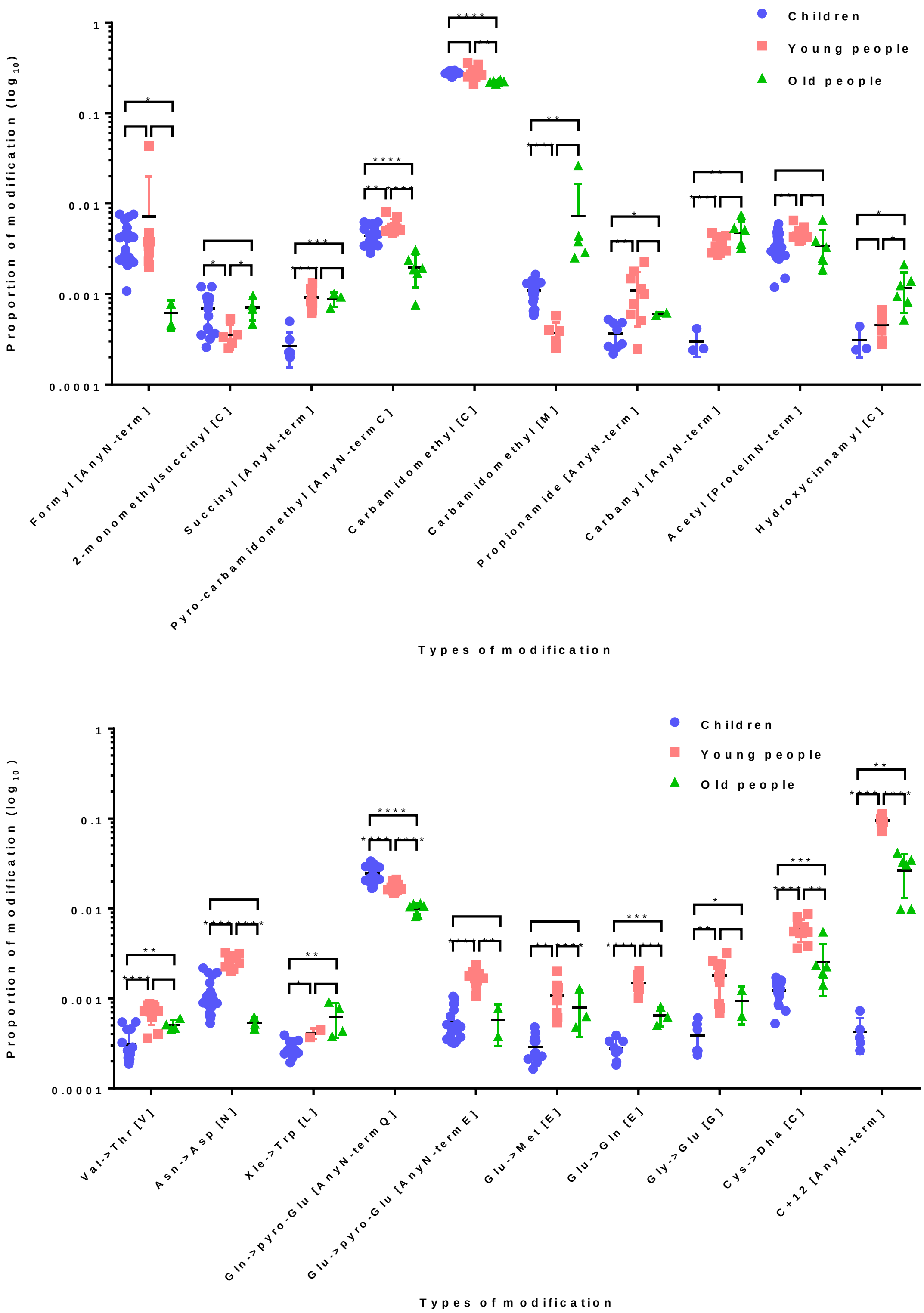


图 4 儿童组、青年组与老年组间共有的修饰差异情况比较。数据中间黑色横线表示中位数，上下彩色线条表示数据四分位范围，数据上方表示组间显著性，随着标识数量的增加表明显著性的提高。

Fig 4 Comparison of modification differences among children group, young people group and old people group. The black horizontal line in the middle of the data represents the median, the upper and lower color lines represent the data quartile range, and the upper part of the data represents the significance between groups. As the number of labels increases, the significance increases.

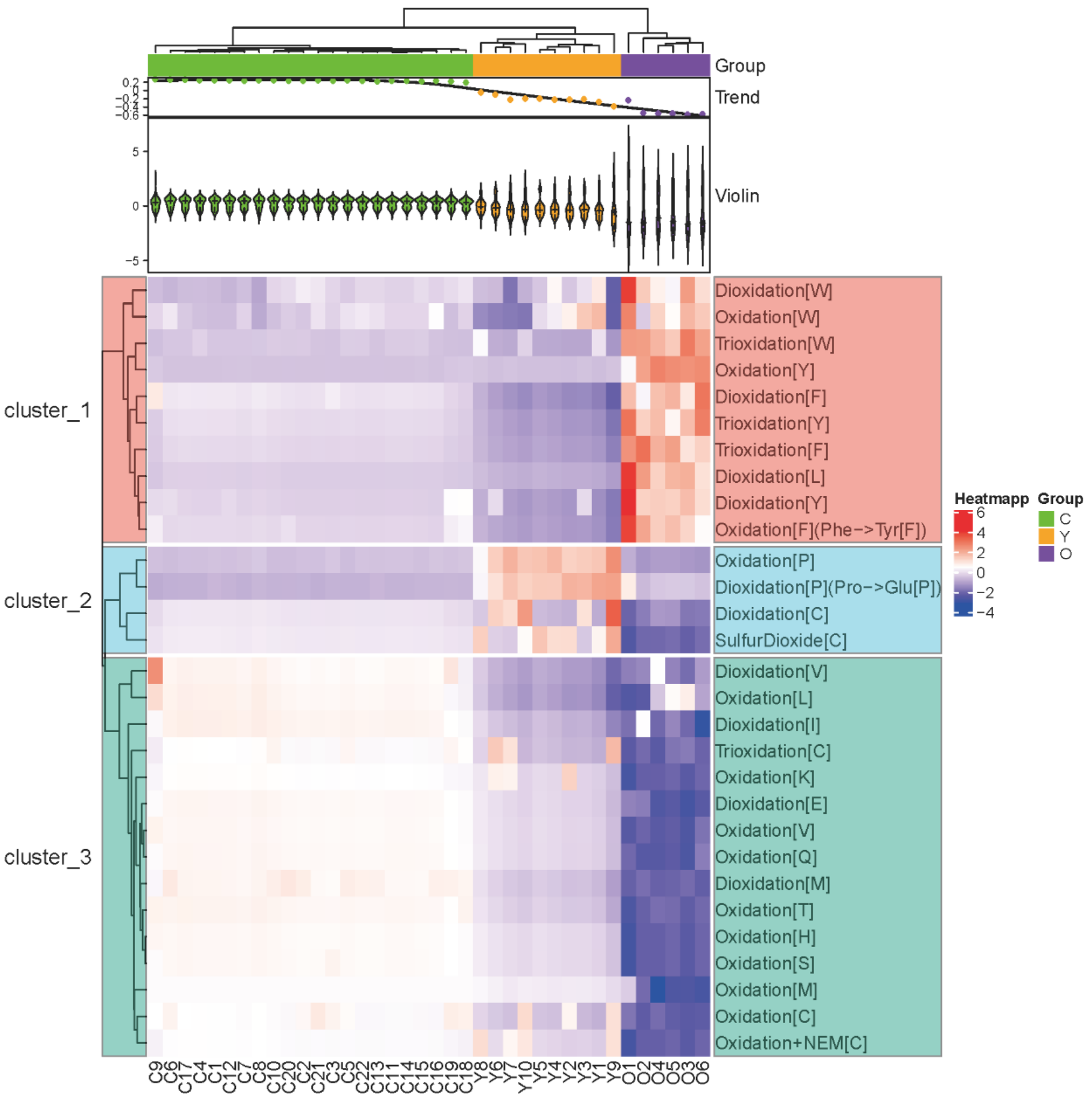
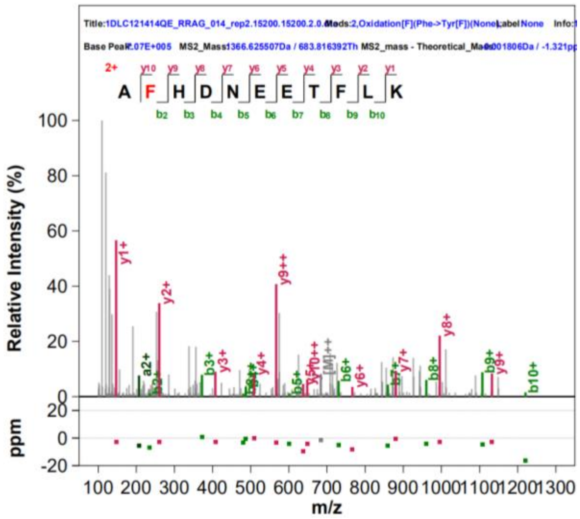


图 5 三组样本中所有氧化修饰的聚类分析结果。绿色为儿童组样本，橙色为青年组样，紫色为老年组样本；

Fig 5 Cluster analysis results of the all oxidative modifications among the three groups of samples. Green is the sample of children group, and orange is the sample of young people group, purple is the sample old people group;

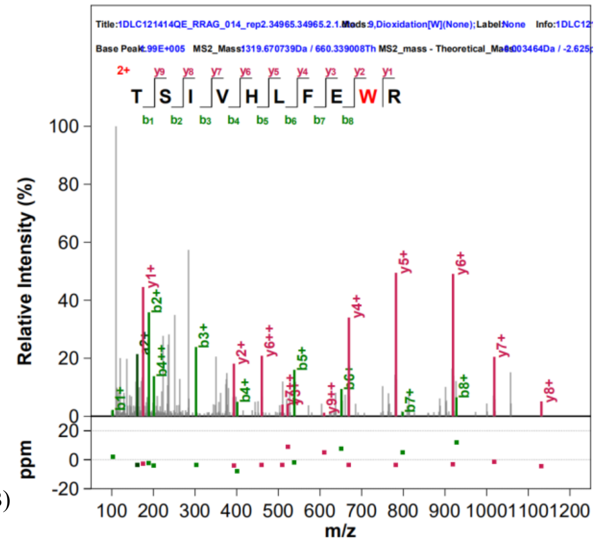
老年组尿液蛋白样本中色氨酸(W)、脯氨酸(P)、半胱氨酸(C)、酪氨酸(Y)等多种氨基酸氧化修饰与丝氨酸(S)羰基化修饰的数量显著高于儿童组及青年组样本，而脲甲基化（IAA 引入的烷基化修饰）修饰显著低于儿童组及青年组样本，由于脲甲基化是作用于还原态二硫键的修饰，这间接说明老年组尿蛋白质中的二硫键完整性较青年组与儿童组显著降低。丝氨酸的磷酸化、半胱氨酸的马来酰化、天冬酰胺(N)替换为天冬氨酸(D)、谷氨酸(E)替换甲硫氨酸(M)与谷氨酰胺(Q)、甘氨酸(G)替换为谷氨酸、半胱氨酸脱巯基成为脱氢丙氨酸(Dha)等修饰青年组要显著高于儿童组与老年组。在儿童组中，蛋白质链中的谷氨酸与天冬氨酸处钙离子相关的修饰要显著高于青年组与老年组，而涉及半胱氨酸的脱氢和蛋白质 N 末端的多种修饰（氨甲酰化、甘氨酰胺化、琥珀酰）的数量却低于青年组与老年组。另外，谷氨酰胺聚合为焦谷氨酸(pyro-Glu)的修饰随年龄增长呈现递减的趋势。

老年组中异亮氨酸(I)、谷氨酰胺、谷氨酸、苯丙氨酸(F)的氧化，天冬氨酸、谷氨酸及酪氨酸的磷酸化是一部分独有的修饰类型（更多信息参见附表 1）。图 6 以老年组样本二级质谱(MS2)结果中几种多肽为例，展示其修饰鉴定信息。



#	b	y
A(*/*)	*	*
F(2/10)	235.1077	1295.5902
H(3/9)	372.1666	1132.5268
D(4/8)	487.1935	995.4679
N(5/7)	601.2364	880.4410
E(6/6)	730.2790	766.3981
E(7/5)	859.3216	637.3555
T(8/4)	960.3693	508.3129
F(9/3)	1107.4377	407.2652
L(10/2)	1220.5217	260.1968
K(*/1)	*	147.1127

(A)



#	b	y
T(1/*)	102.0549	*
S(2/9)	189.0869	1218.6265
I(3/8)	302.1710	1131.5945
V(4/7)	401.2394	1018.5104
H(5/6)	538.2983	919.4420
L(6/5)	651.3824	782.3831
F(7/4)	798.4508	669.2990
E(8/3)	927.4934	522.2306
W(*/2)	*	393.1880
R(*/1)	*	175.1189

(B)

图 6 老年组样本中几种多肽的修饰质谱鉴定结果。(A) α-淀粉酶多肽(Accession:P04745)中色氨酸(Trp)位点的双氧化 b/y 离子信息; (B) 血清白蛋白多肽(Accession:P02768）中苯丙氨酸(Phe)氧化成为酪氨酸(Tyr)的氧化 b/y 离子信息；

Fig 6 Modified identification results of several peptides by MS in samples from the old people group. (A) Alpha-amylase peptide in the tryptophan (Trp) site dioxidation b / y ion information; (B) serum albumin peptide the oxidation b / y ion information of phenylalanine (Phe) oxidized to tyrosine (Tyr);

在三个年龄组各随机选取 1 个样本，在三个样本中查找上述修饰所在的蛋白质，取并集得到 971 种蛋白质，在 WebGestalt（http://www.webgestalt.org）进行蛋白质相互作用分析（PPI BIOGRID—Network Topology-based Analysis(NTA)），默认参数下运行，相关生物过程通路的分析结果表明修饰影响了机体的免疫系统及凝血相关系统的功能，通路图如图 7 所示。其中有 446 种蛋白质中存在以上所鉴定到的五种氧化修饰，占总数 45.9%。甲硫氨酸的氧化可能受到实验干扰，在 UniMod 数据库中一些修饰被定为人工引入，非人工修饰在 359 种蛋白质中存在，其中脯氨酸、色氨酸、酪氨酸、半胱氨酸的氧化修饰在 256 种蛋白质中存在，占比为 71.3%。通过在 pBuild 的 Peptide.all_result，中查寻非人工修饰所属的多肽统计个数，在 pFind.Summary 直接查找非人工修饰所在位点的个数，通过比较与计算，发现在儿童组样本中，被氧化修饰的比例为 12.97%（以多肽计，下同），青年组中氧化修饰的情况为 13.71%，而老年组氧化修饰情况高达

29.76%，显著高于儿童组与青年组，详见表 6。

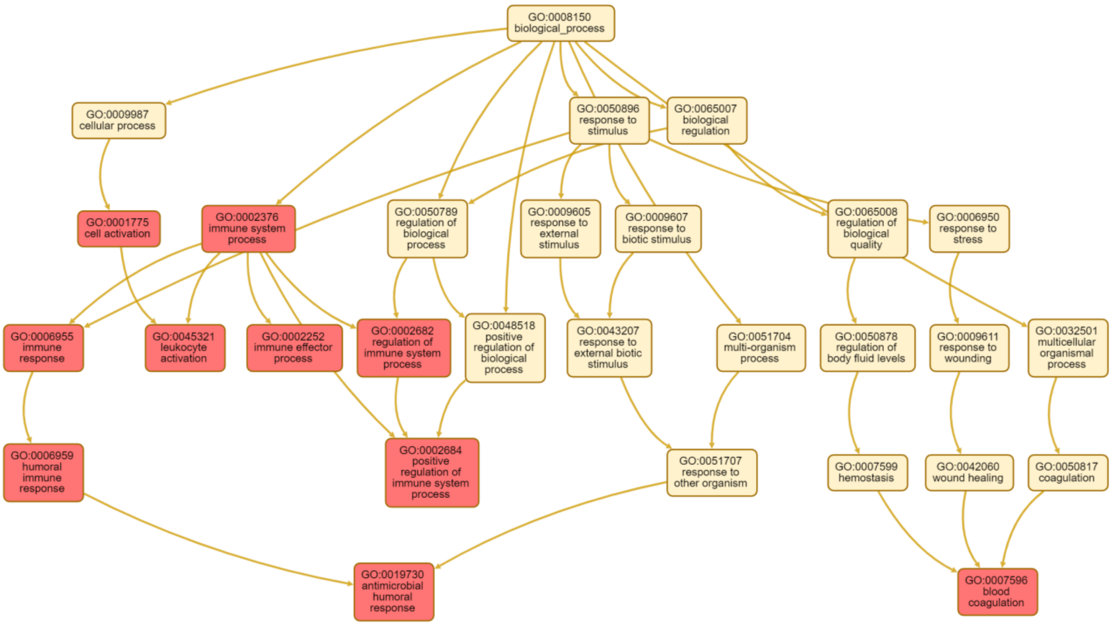


图 7 富集的基因功能项目图

Fig 7 Enriched GO terms graph

表 6 三组样本中非人工修饰多肽及位点情况与统计

Table 6 Statistics of non-artificial modified peptides and sites in samples

	儿童组		青年组		老年组	
	修饰的多肽数	修饰的位点数	修饰的多肽数	修饰的位点数	修饰的多肽数	修饰的位点数
Di-/Oxidation[P]	84	39	119	66	94	54
Tri-/Di-/Oxidation[W]	402	129	687	226	661	227
Di-/Oxidation[Y]	1110	372	1535	510	1478	494
Tri-/Oxidation[C]	91	36	130	49	120	45
glycidamide[AnyN-term]	14	3	8	7	0	0
DiDehydro[C]	35	16	49	19	50	20
Phospho[S]	14	8	23	13	23	10
Cation_Ca[II][E]/Cation_Ca[II][D]	5	3	25	12	23	11
Succinyl[AnyN-term]	7	5	2	2	0	0
Carbamyl[AnyN-term]	58	32	51	27	52	29
Val->Thr[V]	8	4	5	3	5	5
Glu->Met[E]	4	2	5	5	7	5
Glu->Gln[E]	5	3	2	2	4	4
Cys->Dha[C]	25	11	39	21	35	15

C+12[AnyN-term]	147	61	812	292	761	258
Oxidation [P+W+Y+C]	1687	576	2471	851	2353	820
all peptides	13003		18024		7906	
Oxidation [P+W+Y+C]/all	12.97%		13.71%		29.76%	

3. 讨论

通过研究发现，老年组蛋白质多种位点的氧化程度均显著高于青年组与儿童组，可以凭借观察尿液蛋白质组氧化情况来推断尿液是排除人体产生氧化与衰老蛋白质的主要途径，老年人产生氧化的蛋白数量多，使得其排除氧化蛋白质的数量也多。蛋白质的易氧化性与其初级结构和三维结构的特征直接相关，蛋白质的氧化修饰可能导致其结构和功能的破坏 [16,17]。氧化蛋白在体内积累的程度是人体衰老的重要体现，或许可以以此来判断人老龄化程度，并确定抗衰老药物是否有效果。

我们在三组脯氨酸氧化修饰的多肽中发现有所属 Collagen (A0A384MDU2_HUMAN)和 Collagen alpha-1(I) chain preproprotein (H9C5C5_HUMAN)这两种蛋白的多肽。而脯氨酸与羟脯氨酸约占胶原蛋白氨基酸的 1/4 至 1/5，胶原蛋白是细胞外基质的主要成分，以被降解，为细胞的生长和增殖提供氨基酸和能量，炎症和肿瘤进展过程中，胶原蛋白也被发现降解 [18,19]。脯氨酸脱氢酶在某些癌细胞系中过表达与凋亡相关，其机制涉及脯氨酸氧化在细胞中产生 ROS[20,21]，由于脯氨酸脱氢酶过表达也与体内肿瘤形成的减少有关，因此它被认为是 p53 诱导的肿瘤抑制的直接调节因子 [21,22]。与此同时，一些癌细胞可能利用脯氨酸氧化来促进增殖和保护免受压力 [23–25]。由此推断，老年人胶原快速衰老带来了大量的氧化的脯氨酸蛋白，这些氧化后的蛋白质被不断地排进尿里，从而使尿液中含有脯氨酸氧化蛋白的含量显著高于年青人与儿童。

我们在三组的色氨酸、酪氨酸氧化修饰以及蛋氨酸替代谷氨酸修饰的多肽中均发现其多肽所属为 Fibrinogen alpha chain (FIBA_HUMAN)，同时我们在老年人独有修饰中也发现所属纤维蛋白原的多肽。纤维蛋白原是最丰富的血浆蛋白之一，也是经常被引起蛋白质翻译后修饰的试剂靶向的血浆蛋白，是凝血级联反应的最终成员 [26]。被凝血酶激活后 [27–29]，纤维蛋白原转化为纤维蛋白，再聚合成复合纤维蛋白网 [30]。纤维蛋白网与血小板、红细胞和少数白细胞一起，是血栓的主要成分 [31,32]。在生理条件下，血栓可以防止损伤部位的失血，但在病理生理条件下，它可以粘连血管(血栓形成)，并释放到血液中(栓塞)，可能导致中风、心肌梗死等 [33,34]。炎症反应中产生的氧化（位于 α C 结构域的 Met^{476} 的氧化）已被证明会导致纤维蛋白纤维变薄，从而导致更密集的血块，更难被蛋白水解，并带来深静脉血栓形成和肺栓塞的风险 [27,35–37]。

所获得的数据的大部分证据表明，氧化应激增强引起的纤维蛋白原氧化修饰可能与疾病发病机制有关 [35]。血浆纤维蛋白凝块由致密的网状纤维组成，由于纤溶酶原在凝块中的扩散受阻，不易裂解 [36]，可在心血管疾病 [37–40]、慢性炎症 [37]、肝病 [38,39]、糖尿病并发症 [40] 患者中观察到。

色氨酸氧化产物随年龄增长和炎症积累，色氨酸分解产物如犬尿氨酸导致骨质疏松，色氨酸氧化代谢物可能对其积累的组织有不同的影响 [41]。酪氨酸的氧化产物中的双酪氨酸的释放只发生在氧化应激(暴露于 H_2O_2)及其后蛋白质水解，因此，它可以被认为是一个特殊标志，如动脉粥样硬化、急性炎症、全身性细菌感染等和白内障等 [1,42–44]。组氨酸、酪氨酸、色氨酸的氧化已经被证明是对各种活性氧的反应，尽管这些通常被认为是不可逆的修饰，但它们与生物蛋白调节的相关性尚不清楚 [45,46]。

蛋白质中最常见的受可逆氧化还原修饰的位点包括氧化还原活性的过渡金属离子中心(如血红素基团、铁硫蛋白的中心、锌硫蛋白的中心)和氧化敏感性氨基酸侧链(主要是半胱氨酸、硒半胱氨酸和甲硫氨酸残基)。这些位点的氧化修饰可以增强或抑制酶的活性(例如，通过氧化具有催化活性的半胱氨酸)，但也可以导致蛋白质-蛋白质相互作用的改变、亚细胞的转运、蛋白质的转化等 [45]。氧化还原信号的研究领域主要集中在半胱氨酸残基的可逆氧化上，半胱氨酸残基在蛋白质中具有高度的保守性，且随着多细胞生物的进化，半胱氨酸蛋白的相对丰度也在增加 [47,48]。与迅速增长的关于半胱氨酸氧化的文献相比，蛋氨酸氧化作为一种潜在的信号机制还未被充分认识。这在很大程度上是由于缺乏生化试剂来评估甲硫氨酸在生物系统中的氧化，而且只有 MS 方法才能成功地证明甲硫氨酸磺化氧化。甲硫氨酸残基的磺化氧化显著提高了蛋氨酸

的亲水性，从而可以显著改变蛋白质的理化性质，从而增强或抑制蛋白质活性 [49]。此外，与可逆半胱氨酸氧化类似，已经发现酶系统既可以增强又可以逆转蛋氨酸磺化氧化 [50]。

最近的蛋白质组学分析显示，甲硫氨酸氧化倾向于稳定性更高的磷酸化，这表明甲硫氨酸氧化和蛋白磷酸化途径之间存在复杂的关系 [49]。老化与细胞外环境的逐渐氧化有关。人血浆的氧化还原状态，由半胱氨酸和胱氨酸(Cystine)的浓度来定义，随着年龄的增长，其氧化程度越来越高 [51]。半胱氨酸(和不常见的)甲硫氨酸残基的直接氧化是一个主要反应；这通常比 H_2O_2 更快，并导致蛋白质活性和功能的改变。与 H_2O_2 不同的是，H_2O_2 会被保护性酶快速清除，而蛋白质过氧化物只能缓慢清除，分解代谢是主要的命运。虽然蛋白酶体和溶酶体酶以及其他蛋白酶(如线粒体 Lon)对修饰蛋白的周转是有效的，但蛋白质氢过氧化物抑制了这些途径，这可能有助于修饰蛋白在细胞中的积累。现有证据支持蛋白质氧化与多种人类病理之间的联系，但这种联系是否是因果关系仍有待确定 [52]。甲硫氨酸依赖的 TRPV2(transient receptor potential vanilloid 2)氧化还原敏感性，这可能是调节 TRPV2 活性的重要内源性机制，其在巨细胞吞噬作用中共表达发挥关键作用 [53]。

半胱氨酸位点的碘乙酰胺烷基化修饰（脲甲基化修饰）体现出蛋白质链中巯基数量，半胱氨酸中的巯基是参与蛋白质二硫键形成的重要因素，而二硫键数量可以反映出蛋白质空间结构的完整程度。因此，通过比较不同年龄段尿液样本中脲甲基化修饰，则可以了解蛋白质结构的完整程度，通过实验发现，青年组和儿童组间半胱氨酸的脲甲基化修饰差异不显著，而老年组半胱氨酸脲甲基化修饰显著低于青年组与儿童组，表明蛋白质空间结构被破坏程度随着年龄增高而提高。

除了氧化修饰，我们在之前的文章“健康人的尿液中为什么会有蛋白质？[54]”中也探究过半胱氨酸被脱氢丙氨酸取代以及蛋白质 N 端的氨甲酰化修饰。有研究表明，在慢性肝病及慢性肾脏病或糖尿病患者的血液样本中，人血清白蛋白（HSA）中的半胱氨酸 Cys34 修饰可用作氧化应激相关疾病的标志物 [55]。半胱氨酸变成脱氢丙氨酸(Dha)的这个反应是不可逆的，Dha 是一种亲电试剂，在含有 Dha 的多肽中存在一个不饱和的碳-碳双键，因为它在体外蛋白质链中起烷化剂的作用 [56]，许多含有脱氢丙氨酸的肽具有一定的毒性 [57]。因此，发生这种修饰的蛋白质或肽也都具有一定的毒性，我们之前猜测它们在体内停留久了便会对机体产生毒害作用，最好立即将其排出体外。这样便在尿液中会发现了大量含有修饰成为脱氢丙氨酸的蛋白质。氨甲酰化也是一种不可逆的非酶修饰过程，其过程是尿素的分解产物与蛋白质的 N 末端或赖氨酸残基的侧链反应，并与蛋白质老化有关 [58]。赖氨酸氨基甲酰化可以促进金属离子对特定酶活性的配位作用 [59,60]。据报道，尿素水平升高的患者（例如肾病患者）的血浆中氨甲酰化的量显着增加 [61]。

环氧丙氨酸(GA)为丙烯酰胺(AA) 的 DNA 活性代谢物，而丙烯酰胺(AA)是公认的致癌饮食因素之一，其致癌能力和环氧丙氨酸形成 DNA 加合物的能力即在蛋白质 N 端修饰有关 [62–64]。

脊椎动物物种中众多细胞之间复杂的发育程序和信号通路的调控强烈依赖于磷酸化介导的信号通路 [65–70]。在紊乱区域的丝氨酸和苏氨酸磷酸化更有可能是无功能的，在 18 年一项研究中，以人类和小鼠的磷酸化位点的大规模数据为基础，发现与年轻组相比，老年组中的位点更有可能发挥功能并参与信号通路，而丝氨酸磷酸化修饰可能和神经退行性疾病发生有关 [71]。某些特定基因的突变通过丧失蛋白质功能导致神经退化 [72–74]，翻译后修饰可以调节蛋白质的结构、功能，在几种致病蛋白质中，还可以调节毒性。最近的研究已经确定了磷酸化和精氨酸甲基化之间的联系，有证据表明这些修饰在神经退化中起着重要作用，例如亨廷顿蛋白（Huntingtin, Htt）在丝氨酸 421 处的磷酸化减少表明 Protein kinase B (Akt)信号的失调参与了发病 [75,76]。

我们在之前研究中发现血浆样本中，年老组相比于年轻组半胱氨酸的琥珀酰化修饰显著差异上调，在这次研究中也发现蛋白质 N 端的琥珀酰化修饰差异显著。尽管琥珀酰化的研究还处于起步阶段，但数据清楚地表明，琥珀酰化对健康和疾病有广泛的影响，在神经系统和神经系统疾病中提供了代谢和蛋白功能之间的耦合，是细胞整合的关键 [77]。氨基甲酰化是由尿素、异氰酸的分解产物与蛋白质的 N-端或赖氨酸、精氨酸残基的侧链反应，对蛋白质进行非酶修饰的不可逆过程 [78–81]。

缬氨酸被苏氨酸取代和谷氨酸被蛋氨酸取代发生于 tRNA 的错酰化和负载 tRNA 的编辑 [82,83]。先前的研究表明，取代谷氨酸(Glu)在胃分泌酸调节素(Oxyntomodulin, OXM)的 3 位氨基酸位的天然谷氨酰胺(Gln)

可以降低胰高血糖素受体（glucagon receptor, GCGr）的活性，而不影响胰高血糖素样肽 1 受体(glucagon-like peptide 1 receptor, GLP-1r)的活性[84]。

在差异显著的非人工修饰中，氧化修饰占了很大一部分，生物信息学富集分析显示大部分蛋白质和免疫过程有关，也有白细胞激活，血浆凝固等。在之前的研究中发现，在老年人的肌肉中，核糖体蛋白和与能量代谢相关的蛋白，包括与 TCA 循环、线粒体呼吸和糖酵解相关的蛋白，表达不足，而与先天免疫和适应性免疫、蛋白稳定和选择性剪接有关的蛋白表达过多[85,86]。老龄化是一种复杂的现象，老龄化本身是癌症、神经退行性疾病和 2 型糖尿病等年龄相关疾病发展的基础，近年来的科学研究提出了不同的理论，试图解释衰老过程。到目前为止，还没有一个单一的理论能够完全解释衰老的所有方面。从多年来发现的大量证据来看，损害累积理论是最被接受的理论之一。损伤的积累被认为是氧化应激引起的，氧化应激如何促进蛋白质修饰等[87–90]。

蛋白质氧化与很多疾病有关，尤其是衰老相关的疾病。很多证据表明氧化存在于神经退行性疾病，如阿尔茨海默[88,91–99]和帕金森氏病中[100]，其中蛋白质羰基化已在 AD 患者的大脑[101,102]、路易体痴呆患者[103]和帕金森氏病患者的全脑[104]中被证实。此外，蛋白羰基还存在于各种疾病中，如急性/成人呼吸窘迫综合征[105,106]、慢性肺部疾病[107–110]、肌萎缩侧索硬化[111,112]、类风湿性关节炎和青少年慢性关节炎[113,114]、严重脓毒症[115,116]、囊性纤维化[117,118]、白内障[119]、老年性黄斑变性[120]、慢性肾功能衰竭、尿毒症[121–124]、I 型和 II 型糖尿病[125–129]、炎症性肠道疾病[127]、缺血再灌注损伤[130]、系统性淀粉样变性[131]和原发性动脉高血压[132]。年龄相关疾病中的蛋白质氧化更为显著。以上显示了到目前为止已经证实的蛋白质氧化的许多疾病。蛋白质氧化可能是这些疾病的原因或结果，这些疾病几乎影响到所有的器官系统。

翻译后修饰存在于人体内，参与细胞分化和基因调控等多种生理功能。然而，在高浓度时，它们可能预示着严重的疾病，如心肌梗死、静脉血栓栓塞、动脉和静脉血栓形成、肺栓塞和癌症[133–137]。尿液相比于血液来说无创，便于收集，且是一个富集的过程，差异蛋白的变化早于血液，我们对比了三个不同年龄段的修饰，共有的 62 种修饰可以很好的区别开不同年龄组，而其中差异修饰尤其是其中的氧化修饰或许对机体衰老以及相关疾病有一定的早期标志作用。

References

1. Aebersold, R. & Mann, M. Mass-spectrometric exploration of proteome structure and function. *Nature* **537,** 347–355; 10.1038/nature19949 (2016).

2. Sakamoto, S. & Hamachi, I. Recent Progress in Chemical Modification of Proteins. *Analytical sciences : the international journal of the Japan Society for Analytical Chemistry* **35,** 5–27; 10.2116/analsci.18R003 (2019).

3. Wang, C. *et al.* Dynamic modifications of biomacromolecules: mechanism and chemical interventions. *Science China. Life sciences* **62,** 1459–1471; 10.1007/s11427-019-9823-1 (2019).

4. Minguez, P. *et al.* Deciphering a global network of functionally associated post-translational modifications. *Molecular systems biology* **8,** 599; 10.1038/msb.2012.31 (2012).

5. Pieroni, L. *et al.* Enrichments of post-translational modifications in proteomic studies. *Journal of separation science* **43,** 313–336; 10.1002/jssc.201900804 (2020).

6. GOTO, T., KUDO, Y., LEE, S. H. & OE, T. Efficient Strategy for Screening Chemical Modifications on Human Serum Albumin: Use of LC/MS/MS and Differential Analysis Software. *Bunseki kagaku* **64,** 653–659; 10.2116/bunsekikagaku.64.653 (2015).

7. An, Z. *et al.* PTMiner: Localization and Quality Control of Protein Modifications Detected in an Open Search and Its Application to Comprehensive Post-translational Modification Characterization in Human Proteome.

Molecular & cellular proteomics : MCP **18,** 391–405; 10.1074/mcp.RA118.000812 (2019).

8. Chi, H. *et al.* pFind-Alioth: A novel unrestricted database search algorithm to improve the interpretation of high-resolution MS/MS data. *Journal of proteomics* **125,** 89–97; 10.1016/j.jprot.2015.05.009 (2015).
9. Li, D. *et al.* pFind: a novel database-searching software system for automated peptide and protein identification via tandem mass spectrometry. *Bioinformatics (Oxford, England)* **21,** 3049–3050; 10.1093/bioinformatics/bti439 (2005).
10. Wang, L.-H. *et al.* pFind 2.0: a software package for peptide and protein identification via tandem mass spectrometry. *Rapid communications in mass spectrometry : RCM* **21,** 2985–2991; 10.1002/rcm.3173 (2007).
11. Creasy, D. M. & Cottrell, J. S. Unimod: Protein modifications for mass spectrometry. *Proteomics* **4,** 1534–1536; 10.1002/pmic.200300744 (2004).
12. Lehallier, B. *et al.* Undulating changes in human plasma proteome profiles across the lifespan. *Nature medicine* **25,** 1843–1850; 10.1038/s41591-019-0673-2 (2019).
13. Gao, Y. & Li, X. *The potential urinary aging markers of 20-month-old rats* (2015).
14. Yuan, Y. & Gao, Y. Urinary Protein Changes in A Rat Starvation Model. *MOJPB* **6**; 10.15406/mojpb.2017.06.00202 (2017).
15. Wiśniewski, J. R., Zougman, A., Nagaraj, N. & Mann, M. Universal sample preparation method for proteome analysis. *Nature methods* **6,** 359–362; 10.1038/nmeth.1322 (2009).
16. Rosenfeld, M. A., Vasilyeva, A. D., Yurina, L. V. & Bychkova, A. V. Oxidation of proteins: is it a programmed process? *Free radical research* **52,** 14–38; 10.1080/10715762.2017.1402305 (2018).
17. Sovová, Ž. *et al.* Impact of posttranslational modifications on atomistic structure of fibrinogen. *PloS one* **15,** e0227543; 10.1371/journal.pone.0227543 (2020).
18. Bella, J., Eaton, M., Brodsky, B. & Berman, H. M. Crystal and molecular structure of a collagen-like peptide at 1.9 A resolution. *Science (New York, N.Y.)* **266,** 75–81; 10.1126/science.7695699 (1994).
19. Phang, J. M., Pandhare, J. & Liu, Y. The metabolism of proline as microenvironmental stress substrate. *The Journal of nutrition* **138,** 2008S-2015S; 10.1093/jn/138.10.2008S (2008).
20. Maxwell, S. A. & Rivera, A. Proline oxidase induces apoptosis in tumor cells, and its expression is frequently absent or reduced in renal carcinomas. *The Journal of biological chemistry* **278,** 9784–9789; 10.1074/jbc.M210012200 (2003).
21. Donald, S. P. *et al.* Proline oxidase, encoded by p53-induced gene-6, catalyzes the generation of proline-dependent reactive oxygen species. *Cancer research* **61,** 1810–1815 (2001).
22. Liu, Y. *et al.* Proline oxidase functions as a mitochondrial tumor suppressor in human cancers. *Cancer research* **69,** 6414–6422; 10.1158/0008-5472.CAN-09-1223 (2009).
23. Liu, W. *et al.* Proline oxidase promotes tumor cell survival in hypoxic tumor microenvironments. *Cancer research* **72,** 3677–3686; 10.1158/0008-5472.CAN-12-0080 (2012).
24. Natarajan, S. K. *et al.* Proline dehydrogenase is essential for proline protection against hydrogen peroxide-induced cell death. *Free radical biology & medicine* **53,** 1181–1191; 10.1016/j.freeradbiomed.2012.07.002 (2012).

25. Pandhare, J., Donald, S. P., Cooper, S. K. & Phang, J. M. Regulation and function of proline oxidase under nutrient stress. *Journal of cellular biochemistry* **107,** 759–768; 10.1002/jcb.22174 (2009).

26. Shacter, E., Williams, J. A., Lim, M. & Levine, R. L. Differential susceptibility of plasma proteins to oxidative modification: examination by western blot immunoassay. *Free Radical Biology and Medicine* **17,** 429–437; 10.1016/0891-5849(94)90169-4 (1994).

27. BAILEY, K., BETTELHEIM, F. R., LORAND, L. & MIDDLEBROOK, W. R. Action of thrombin in the clotting of fibrinogen. *Nature* **167,** 233–234; 10.1038/167233a0 (1951).

28. LORAND, L. 'Fibrino-peptide'; new aspects of the fibrinogen-fibrin transformation. *Nature* **167,** 992–993; 10.1038/167992a0 (1951).

29. Pederson, E. N. & Interlandi, G. Oxidation-induced destabilization of the fibrinogen αC-domain dimer investigated by molecular dynamics simulations. *Proteins* **87,** 826–836; 10.1002/prot.25746 (2019).

30. Weisel, J. W. & Litvinov, R. I. Fibrin Formation, Structure and Properties. *Sub-cellular biochemistry* **82,** 405–456; 10.1007/978-3-319-49674-0_13 (2017).

31. Sadowski, M., Ząbczyk, M. & Undas, A. Coronary thrombus composition: links with inflammation, platelet and endothelial markers. *Atherosclerosis* **237,** 555–561; 10.1016/j.atherosclerosis.2014.10.020 (2014).

32. Silvain, J. *et al.* Composition of coronary thrombus in acute myocardial infarction. *Journal of the American College of Cardiology* **57,** 1359–1367; 10.1016/j.jacc.2010.09.077 (2011).

33. Undas, A. & Ariëns, R. A. S. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. *Arteriosclerosis, thrombosis, and vascular biology* **31,** e88-99; 10.1161/ATVBAHA.111.230631 (2011).

34. Ząbczyk, M. & Undas, A. Plasma fibrin clot structure and thromboembolism: clinical implications. *Polish archives of internal medicine* **127,** 873–881; 10.20452/pamw.4165 (2017).

35. Martinez, M., Weisel, J. W. & Ischiropoulos, H. Functional impact of oxidative posttranslational modifications on fibrinogen and fibrin clots. *Free radical biology & medicine* **65,** 411–418; 10.1016/j.freeradbiomed.2013.06.039 (2013).

36. Undas, A. Fibrin clot properties and their modulation in thrombotic disorders. *Thrombosis and haemostasis* **112,** 32–42; 10.1160/TH14-01-0032 (2014).

37. Torbitz, V. D. *et al.* In vitro oxidation of fibrinogen promotes functional alterations and formation of advanced oxidation protein products, an inflammation mediator. *Inflammation* **38,** 1201–1206; 10.1007/s10753-014-0085-x (2015).

38. Lisman, T. & Ariëns, R. A. S. Alterations in Fibrin Structure in Patients with Liver Diseases. *Seminars in thrombosis and hemostasis* **42,** 389–396; 10.1055/s-0036-1572327 (2016).

39. Hugenholtz, G. C. G. *et al.* Procoagulant changes in fibrin clot structure in patients with cirrhosis are associated with oxidative modifications of fibrinogen. *Journal of thrombosis and haemostasis : JTH* **14,** 1054–1066; 10.1111/jth.13278 (2016).

40. Lados-Krupa, A., Konieczynska, M., Chmiel, A. & Undas, A. Increased Oxidation as an Additional Mechanism Underlying Reduced Clot Permeability and Impaired Fibrinolysis in Type 2 Diabetes. *Journal of diabetes research* **2015,** 456189; 10.1155/2015/456189 (2015).

41. Ding, K. *et al.* Picolinic acid, a tryptophan oxidation product, does not impact bone mineral density but increases marrow adiposity. *Experimental gerontology* **133,** 110885 (2020).

42. Giulivi, C., Traaseth, N. J. & Davies, K. J. A. Tyrosine oxidation products: analysis and biological relevance. *Amino acids* **25,** 227–232; 10.1007/s00726-003-0013-0 (2003).

43. Garcia-Castineiras, S., Dillon, J. & Spector, A. Detection of bityrosine in cataractous human lens protein. *Science (New York, N.Y.)* **199,** 897–899; 10.1126/science.622574 (1978).

44. Heinecke, J. W. Oxidized amino acids: culprits in human atherosclerosis and indicators of oxidative stress 1,2 1This article is part of a series of reviews on "Oxidatively Modified Proteins in Aging and Disease." The full list of papers may be found on the homepage of the journal. 2Guest Editor: Earl Stadtman. *Free Radical Biology and Medicine* **32,** 1090–1101; 10.1016/S0891-5849(02)00792-X (2002).

45. van der Vliet, A., Janssen-Heininger, Y. M. W. & Anathy, V. Oxidative stress in chronic lung disease: From mitochondrial dysfunction to dysregulated redox signaling. *Molecular aspects of medicine* **63,** 59–69; 10.1016/j.mam.2018.08.001 (2018).

46. Dustin, C. M., Heppner, D. E., Lin, M.-C. J. & van der Vliet, A. Redox regulation of tyrosine kinase signalling: more than meets the eye. *Journal of biochemistry* **167,** 151–163; 10.1093/jb/mvz085 (2020).

47. van der Vliet, A. NADPH oxidases in lung biology and pathology: host defense enzymes, and more. *Free Radical Biology and Medicine* **44,** 938–955; 10.1016/j.freeradbiomed.2007.11.016 (2008).

48. Jones, D. P. & Sies, H. The Redox Code. *Antioxidants & redox signaling* **23,** 734–746; 10.1089/ars.2015.6247 (2015).

49. Veredas, F. J., Cantón, F. R. & Aledo, J. C. Methionine residues around phosphorylation sites are preferentially oxidized in vivo under stress conditions. *Scientific Reports* **7,** 40403 (2017).

50. Manta, B. & Gladyshev, V. N. Regulated methionine oxidation by monooxygenases. *Free radical biology & medicine* **109,** 141–155; 10.1016/j.freeradbiomed.2017.02.010 (2017).

51. Zheng, Y. *et al.* Age-dependent oxidation of extracellular cysteine/cystine redox state (Eh(Cys/CySS)) in mouse lung fibroblasts is mediated by a decline in Slc7a11 expression. *Free radical biology & medicine* **118,** 13–22; 10.1016/j.freeradbiomed.2018.02.026 (2018).

52. Davies, M. J. Protein oxidation and peroxidation. *The Biochemical journal* **473,** 805–825; 10.1042/BJ20151227 (2016).

53. Fricke, T. C. *et al.* Oxidation of methionine residues activates the high-threshold heat-sensitive ion channel TRPV2. *Proceedings of the National Academy of Sciences of the United States of America* **116,** 24359–24365; 10.1073/pnas.1904332116 (2019).

54. ZHAO, M., PAN, X., Gao, Y. & LIU, Y. Why are there proteins in the urine of healthy people? *Sci. Sin.-Vitae* **50,** 338–348; 10.1360/SSV-2019-0235 (2020).

55. Bechtel, T. J. & Weerapana, E. From structure to redox: The diverse functional roles of disulfides and implications in disease. *Proteomics* **17**; 10.1002/pmic.201600391 (2017).

56. Friedman, M. Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *Journal of agricultural and food chemistry* **47,** 1295–1319; 10.1021/jf981000+ (1999).

57. Siodłak, D. α,β-Dehydroamino acids in naturally occurring peptides. *Amino acids* **47,** 1–17; 10.1007/s00726-014-1846-4 (2015).

58. Kollipara, L. & Zahedi, R. P. Protein carbamylation: in vivo modification or in vitro artefact? *Proteomics* **13,** 941–944; 10.1002/pmic.201200452 (2013).

59. Badar, A., Arif, Z. & Alam, K. Role of Carbamylated Biomolecules in Human Diseases. *IUBMB life* **70,** 267–275; 10.1002/iub.1732 (2018).

60. Hsieh, Y.-C. *et al.* Crystal structures of vertebrate dihydropyrimidinase and complexes from Tetraodon nigroviridis with lysine carbamylation: metal and structural requirements for post-translational modification and function. *The Journal of biological chemistry* **288,** 30645–30658; 10.1074/jbc.M113.496778 (2013).

61. Shi, J. *et al.* Carbamylation and antibodies against carbamylated proteins in autoimmunity and other pathologies. *Autoimmunity reviews* **13,** 225–230; 10.1016/j.autrev.2013.10.008 (2014).

62. Besaratinia, A. & Pfeifer, G. P. Genotoxicity of acrylamide and glycidamide. *Journal of the National Cancer Institute* **96,** 1023–1029; 10.1093/jnci/djh186 (2004).

63. Feng, C.-H. & Lu, C.-Y. Modification of major plasma proteins by acrylamide and glycidamide: Preliminary screening by nano liquid chromatography with tandem mass spectrometry. *Analytica chimica acta* **684,** 80–86; 10.1016/j.aca.2010.10.042 (2011).

64. Lima, J. P. de, Silva, S. N., Rueff, J. & Pingarilho, M. Glycidamide genotoxicity modulated by Caspases genes polymorphisms. *Toxicology in vitro : an international journal published in association with BIBRA* **34,** 123–127; 10.1016/j.tiv.2016.03.018 (2016).

65. Hardie, D. G. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nature reviews. Molecular cell biology* **8,** 774–785; 10.1038/nrm2249 (2007).

66. Johnson, L. N. & Barford, D. The effects of phosphorylation on the structure and function of proteins. *Annual review of biophysics and biomolecular structure* **22,** 199–232; 10.1146/annurev.bb.22.060193.001215 (1993).

67. Johnson, L. N. The regulation of protein phosphorylation. *Biochemical Society transactions* **37,** 627–641; 10.1042/BST0370627 (2009).

68. Karin, M. Signal transduction from the cell surface to the nucleus through the phosphorylation of transcription factors. *Current Opinion in Cell Biology* **6,** 415–424; 10.1016/0955-0674(94)90035-3 (1994).

69. Pawson, T. & Scott, J. D. Protein phosphorylation in signaling--50 years and counting. *Trends in biochemical sciences* **30,** 286–290; 10.1016/j.tibs.2005.04.013 (2005).

70. Ubersax, J. A. & Ferrell, J. E. Mechanisms of specificity in protein phosphorylation. *Nature reviews. Molecular cell biology* **8,** 530–541; 10.1038/nrm2203 (2007).

71. Miao, B., Xiao, Q., Chen, W., Li, Y. & Wang, Z. Evaluation of functionality for serine and threonine phosphorylation with different evolutionary ages in human and mouse. *BMC genomics* **19,** 431; 10.1186/s12864-018-4661-6 (2018).

72. Keryer, G. *et al.* Ciliogenesis is regulated by a huntingtin-HAP1-PCM1 pathway and is altered in Huntington disease. *The Journal of clinical investigation* **121,** 4372–4382; 10.1172/JCI57552 (2011).

73. Lim, J. *et al.* Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. *Nature* **452,** 713–718; 10.1038/nature06731 (2008).

74. Zuccato, C. *et al.* Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science (New York, N.Y.)* **293,** 493–498; 10.1126/science.1059581 (2001).

75. Basso, M. & Pennuto, M. Serine phosphorylation and arginine methylation at the crossroads to neurodegeneration. *Experimental neurology* **271,** 77–83; 10.1016/j.expneurol.2015.05.003 (2015).

76. Warby, S. C. *et al.* Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo. *Human molecular genetics* **14,** 1569–1577; 10.1093/hmg/ddi165 (2005).

77. Yang, Y. & Gibson, G. E. Succinylation Links Metabolism to Protein Functions. *Neurochemical research* **44,** 2346–2359; 10.1007/s11064-019-02780-x (2019).

78. *Enzyme Structure, Part B* (Elsevier, 1972).

79. Kairaitis, L. K., Yuill, E. & Harris, D. C. Determinants of haemoglobin carbamylation in haemodialysis and peritoneal dialysis patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **15,** 1431–1437; 10.1093/ndt/15.9.1431 (2000).

80. Stark, G. R. in *Enzyme Structure, Part B* (Elsevier1972), pp. 579–584.

81. van Driessche, G., Vandenberghe, I., Jacquemotte, F., Devreese, B. & van Beeumen, J. J. Mass spectrometric identification of in vivo carbamylation of the amino terminus of Ectothiorhodospira mobilis high-potential iron-sulfur protein, isozyme 1. *Journal of mass spectrometry : JMS* **37,** 858–866; 10.1002/jms.348 (2002).

82. Lin, H.-L., Kent, U. M., Zhang, H., Waskell, L. & Hollenberg, P. F. The functional role of threonine-205 in the mechanism-based inactivation of P450 2B1 by two ethynyl substrates: the importance of the F helix in catalysis. *The Journal of pharmacology and experimental therapeutics* **311,** 855–863; 10.1124/jpet.104.071670 (2004).

83. Mohanta, T. K., Syed, A. S., Ameen, F. & Bae, H. Novel Genomic and Evolutionary Perspective of Cyanobacterial tRNAs. *Frontiers in genetics* **8,** 200; 10.3389/fgene.2017.00200 (2017).

84. Price, S. L., Minnion, J. S. & Bloom, S. R. Investigating the Glucagon Receptor and Glucagon-Like Peptide 1 Receptor Activity of Oxyntomodulin-Like Analogues in Male Wistar Rats. *Current therapeutic research, clinical and experimental* **77,** 111–115; 10.1016/j.curtheres.2015.10.003 (2015).

85. Bakun, M. *et al.* Urine proteomes of healthy aging humans reveal extracellular matrix (ECM) alterations and immune system dysfunction. *Age (Dordrecht, Netherlands)* **36,** 299–311; 10.1007/s11357-013-9562-7 (2014).

86. Ubaida-Mohien, C. *et al.* Discovery proteomics in aging human skeletal muscle finds change in spliceosome, immunity, proteostasis and mitochondria. *eLife* **8**; 10.7554/eLife.49874 (2019).

87. Höhn, A. *et al.* Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. *Redox biology* **11,** 482–501; 10.1016/j.redox.2016.12.001 (2017).

88. Aksenov, M., Aksenova, M., Butterfield, D. A. & Markesbery, W. R. Oxidative modification of creatine kinase BB in Alzheimer's disease brain. *Journal of neurochemistry* **74,** 2520–2527; 10.1046/j.1471-4159.2000.0742520.x (2000).

89. HARMAN, D. Aging: a theory based on free radical and radiation chemistry. *Journal of gerontology* **11,** 298–300; 10.1093/geronj/11.3.298 (1956).

90. Miranda-Vizuete, A. & Veal, E. A. Caenorhabditis elegans as a model for understanding ROS function in

physiology and disease. *Redox biology* **11,** 708–714; 10.1016/j.redox.2016.12.020 (2017).

91. Aksenov, M. Y., Aksenova, M. V., Butterfield, D. A., Geddes, J. W. & Markesbery, W. R. Protein oxidation in the brain in Alzheimer's disease. *Neuroscience* **103,** 373–383; 10.1016/s0306-4522(00)00580-7 (2001).

92. Butterfield, D.A. & Lauderback, C. M. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress 1,2 1Guest Editors: Mark A. Smith and George Perry 2This article is part of a series of reviews on "Causes and Consequences of Oxidative Stress in Alzheimer's Disease." The full list of papers may be found on the homepage of the journal. *Free Radical Biology and Medicine* **32,** 1050–1060; 10.1016/s0891-5849(02)00794-3 (2002).

93. Castegna, A. *et al.* Proteomic identification of oxidatively modified proteins in alzheimer's disease brain. part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radical Biology and Medicine* **33,** 562–571; 10.1016/s0891-5849(02)00914-0 (2002).

94. Castegna, A. *et al.* Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *Journal of neurochemistry* **82,** 1524–1532; 10.1046/j.1471-4159.2002.01103.x (2002).

95. Choi, J., Malakowsky, C. A., Talent, J. M., Conrad, C. C. & Gracy, R. W. Identification of oxidized plasma proteins in Alzheimer's disease. *Biochemical and biophysical research communications* **293,** 1566–1570; 10.1016/S0006-291X(02)00420-5 (2002).

96. Conrad, C. C. *et al.* Oxidized proteins in Alzheimer's plasma. *Biochemical and biophysical research communications* **275,** 678–681; 10.1006/bbrc.2000.3356 (2000).

97. Hensley, K. *et al.* Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *Journal of neurochemistry* **65,** 2146–2156; 10.1046/j.1471-4159.1995.65052146.x (1995).

98. Korolainen, M. A., Goldsteins, G., Alafuzoff, I., Koistinaho, J. & Pirttilä, T. *Electrophoresis* **23,** 3428–3433; 10.1002/1522-2683(200210)23:19<3428::AID-ELPS3428>3.0.CO;2-5 (2002).

99. Smith, C. D. *et al.* Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **88,** 10540–10543; 10.1073/pnas.88.23.10540 (1991).

100. Floor, E. & Wetzel, M. G. Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. *Journal of neurochemistry* **70,** 268–275; 10.1046/j.1471-4159.1998.70010268.x (1998).

101. Markesbery, W. R. Oxidative Stress Hypothesis in Alzheimer's Disease. *Free Radical Biology and Medicine* **23,** 134–147; 10.1016/s0891-5849(96)00629-6 (1997).

102. Pamplona, R. *et al.* Proteins in human brain cortex are modified by oxidation, glycoxidation, and lipoxidation. Effects of Alzheimer disease and identification of lipoxidation targets. *The Journal of biological chemistry* **280,** 21522–21530; 10.1074/jbc.M502255200 (2005).

103. Lyras, L. *et al.* Oxidative damage to proteins, lipids, and DNA in cortical brain regions from patients with dementia with Lewy bodies. *Journal of neurochemistry* **71,** 302–312; 10.1046/j.1471-4159.1998.71010302.x (1998).

104. Alam, Z. I. *et al.* A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. *Journal of neurochemistry* **69,** 1326–1329; 10.1046/j.1471-4159.1997.69031326.x (1997).

105. Lenz, A. G. *et al.* Oxidatively modified proteins in bronchoalveolar lavage fluid of patients with ARDS and patients at-risk for ARDS. *The European respiratory journal* **13,** 169–174; 10.1034/j.1399-3003.1999.13a31.x (1999).

106. Quinlan, G. J., Evans, T. W. & Gutteridge, J. M. Oxidative damage to plasma proteins in adult respiratory distress syndrome. *Free radical research* **20,** 289–298; 10.3109/10715769409145628 (1994).

107. Buss, I. H., Darlow, B. A. & Winterbourn, C. C. Elevated protein carbonyls and lipid peroxidation products correlating with myeloperoxidase in tracheal aspirates from premature infants. *Pediatric research* **47,** 640–645; 10.1203/00006450-200005000-00014 (2000).

108. Gladstone, I. M. & Levine, R. L. Oxidation of proteins in neonatal lungs. *Pediatrics* **93,** 764–768 (1994).

109. Ramsay, P. L. *et al.* Clara cell secretory protein oxidation and expression in premature infants who develop bronchopulmonary dysplasia. *American journal of respiratory and critical care medicine* **164,** 155–161; 10.1164/ajrccm.164.1.2008022 (2001).

110. Schock, B. C., Sweet, D. G., Halliday, H. L., Young, I. S. & Ennis, M. Oxidative stress in lavage fluid of preterm infants at risk of chronic lung disease. *American journal of physiology. Lung cellular and molecular physiology* **281,** L1386-91; 10.1152/ajplung.2001.281.6.L1386 (2001).

111. Ferrante, R. J. *et al.* Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *Journal of neurochemistry* **69,** 2064–2074; 10.1046/j.1471-4159.1997.69052064.x (1997).

112. Siciliano, G. *et al.* Antioxidant capacity and protein oxidation in cerebrospinal fluid of amyotrophic lateral sclerosis. *Journal of neurology* **254,** 575–580; 10.1007/s00415-006-0301-1 (2007).

113. Mantle, D., Falkous, G. & Walker, D. Quantification of protease activities in synovial fluid from rheumatoid and osteoarthritis cases: comparison with antioxidant and free radical damage markers. *Clinica Chimica Acta* **284,** 45–58; 10.1016/s0009-8981(99)00055-8 (1999).

114. Renke, J., Popadiuk, S., Korzon, M., Bugajczyk, B. & Woźniak, M. Protein carbonyl groups' content as a useful clinical marker of antioxidant barrier impairment in plasma of children with juvenile chronic arthritis. *Free Radical Biology and Medicine* **29,** 101–104; 10.1016/s0891-5849(00)00288-4 (2000).

115. Abu-Zidan, F. M., Plank, L. D. & Windsor, J. A. Proteolysis in severe sepsis is related to oxidation of plasma protein. *The European journal of surgery = Acta chirurgica* **168,** 119–123; 10.1080/11024150252884359 (2002).

116. Winterbourn, C. C. *et al.* Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. *Critical care medicine* **28,** 143–149; 10.1097/00003246-200001000-00024 (2000).

117. McGrath, L. T. *et al.* Oxidative stress during acute respiratory exacerbations in cystic fibrosis. *Thorax* **54,** 518–523; 10.1136/thx.54.6.518 (1999).

118. Range, S. P., Dunster, C., Knox, A. J. & Kelly, F. J. Treatment of pulmonary exacerbations of cystic fibrosis leads to improved antioxidant status. *The European respiratory journal* **13,** 560–564; 10.1183/09031936.99.13356099 (1999).

119. Boscia, F., Grattagliano, I., Vendemiale, G., Micelli-Ferrari, T. & Altomare, E. Protein oxidation and lens

opacity in humans. *Investigative ophthalmology & visual science* **41,** 2461–2465 (2000).

120. Totan, Y. *et al.* Oxidative macromolecular damage in age-related macular degeneration. *Current eye research* **34,** 1089–1093; 10.3109/02713680903353772 (2009).

121. Himmelfarb, J. & McMonagle, E. Albumin is the major plasma protein target of oxidant stress in uremia. *Kidney international* **60,** 358–363; 10.1046/j.1523-1755.2001.00807.x (2001).

122. Himmelfarb, J., McMonagle, E. & McMenamin, E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney international* **58,** 2571–2578; 10.1046/j.1523-1755.2000.00443.x (2000).

123. Lim, P.-S., Cheng, Y.-M. & Wei, Y.-H. Increase in oxidative damage to lipids and proteins in skeletal muscle of uremic patients. *Free radical research* **36,** 295–301; 10.1080/10715760290019318 (2002).

124. Miyata, T., van Ypersele de Strihou, C., Kurokawa, K. & Baynes, J. W. Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. *Kidney international* **55,** 389–399; 10.1046/j.1523-1755.1999.00302.x (1999).

125. Cakatay, U., Telci, A., Salman, S., Satman, L. & Sivas, A. Oxidative protein damage in type I diabetic patients with and without complications. *Endocrine research* **26,** 365–379; 10.3109/07435800009066174 (2000).

126. Domínguez, C., Ruiz, E., Gussinye, M. & Carrascosa, A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. *Diabetes care* **21,** 1736–1742; 10.2337/diacare.21.10.1736 (1998).

127. Grattagliano, I. *et al.* Oxidative Retinal Products and Ocular Damages in Diabetic Patients. *Free Radical Biology and Medicine* **25,** 369–372; 10.1016/s0891-5849(98)00059-8 (1998).

128. Telci, A., Çakatay, U., Salman, S., Satman, İ. & Sivas, A. Oxidative protein damage in early stage Type 1 diabetic patients. *Diabetes Research and Clinical Practice* **50,** 213–223; 10.1016/s0168-8227(00)00197-2 (2000).

129. Telci, A. *et al.* Oxidative protein damage in plasma of type 2 diabetic patients. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* **32,** 40–43; 10.1055/s-2007-978584 (2000).

130. Pantke, U. *et al.* Oxidized proteins as a marker of oxidative stress during coronary heart surgery. *Free Radical Biology and Medicine* **27,** 1080–1086; 10.1016/s0891-5849(99)00144-6 (1999).

131. Miyata, T., Ueda, Y., Saito, A. & Kurokawa, K. 'Carbonyl stress' and dialysis-related amyloidosis. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **15 Suppl 1,** 25–28; 10.1093/oxfordjournals.ndt.a027959 (2000).

132. Kedziora-Kornatowska, K. *et al.* The markers of oxidative stress and activity of the antioxidant system in the blood of elderly patients with essential arterial hypertension. *Cellular & molecular biology letters* **9,** 635–641 (2004).

133. Pignatelli, B. *et al.* Nitrated and oxidized plasma proteins in smokers and lung cancer patients. *Cancer research* **61,** 778–784 (2001).

134. Vadseth, C. *et al.* Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species. *The Journal of biological chemistry* **279,** 8820–8826; 10.1074/jbc.M306101200 (2004).

135. Mitchell, A. M., Nordenholz, K. E. & Kline, J. A. Tandem measurement of D-dimer and myeloperoxidase or C-reactive protein to effectively screen for pulmonary embolism in the emergency department. *Academic*

emergency medicine : official journal of the Society for Academic Emergency Medicine **15,** 800–805; 10.1111/j.1553-2712.2008.00204.x (2008).

136. Halliwell, B. Oxidative stress and cancer: have we moved forward? *The Biochemical journal* **401,** 1–11; 10.1042/BJ20061131 (2007).

137. Martinez, M. *et al.* Nitrated fibrinogen is a biomarker of oxidative stress in venous thromboembolism. *Free radical biology & medicine* **53,** 230–236; 10.1016/j.freeradbiomed.2012.05.004 (2012).

附表 1 不同组间样品修饰类型交集的韦恩图信息

Appendix Table 1 Venn diagram information of the intersection of sample modification types between different groups

Intersection between groups	Total	Modification types
Children & Old People Young People	62	AzidoF[F] Carbamyl[C] Hex[K] Xle->Pro[L] Formyl[AnyN-term] Cation_Na[E] Phospho[S] Carboxymethyl[C] Carboxymethyl_13C(2)[C] Dioxidation[W] Pro->Trp[P] Deamidated[Q] Dioxidation[Y] Arg-loss[AnyC-termR] Oxidation[W] Ammonium[E] Glu->Met[E] Oxidation[C] Carbonyl[S] Deamidated[N] Carbamidomethyl[M] Xle->Asn[L] Cation_Ca[II][D] maleimide[C] HexNAc[T] Propionamide_2H(3)[C] Dioxidation[P](Pro->Glu[P]) Propionamide[AnyN-term] Gln->pyro-Glu[AnyN-termQ] Pyro-carbamidomethyl[AnyN-termC] 2-monomethylsuccinyl[C] C+12[AnyN-term] Gly->Glu[G] Oxidation[P] Ammonium[D] Hydroxycinnamyl[C] Glu->Asp[E] GG[C](Dicarbamidomethyl[C]) Glu->Gln[E] Acetyl[ProteinN-term] Gly->Met[G] Carbamidomethyl[C] Xle->Trp[L] Diethylphosphate[C] Carbamidomethyl[AnyN-term] Cation_Na[D] Trioxidation[C] Trioxidation[W] Cys->Dha[C] sulfo+amino[Y] Ammonia-loss[N] Asn->Trp[N] Carbamyl[AnyN-term] Oxidation[Y] Succinyl[AnyN-term] DiDehydro[C] Cation_Ca[II][E] Glu->pyro-Glu[AnyN-termE] Val->Thr[V] Oxidation[M] Met->AspSA[M] glycidamide[AnyN-term]
Children & Young People	101	Hex(2)[S] Cys->Ser[C] Cation_Fe[III][E] Asn->His[N] Thr->Ser[T] HexNAc[N] Acetyl[AnyN-term] Hex[T] Methyl[AnyN-term] methylol[W] Xle->Asn[I] Methyl_2H(3)13C(1)[AnyN-term] Cation_Na[AnyC-term] Unknown_250[AnyN-term] Pro->Val[P] AEC-MAEC[T] Dehydrated[D] Xlink_SMCC[219][C] Hex(1)HexNAc(1)Phos(1)[S] Thr->Xle[T] Delta_H(4)C(3)O(1)[C] Propyl[AnyN-term] Cation_Ni[II][E] Menadione[C] dHex(1)Hex(2)[T] Val->Ala[V] Ala->Asp[A] Dehydrated[T] Thr->Asn[T] Ala->Val[A] Dehydrated[S] Carboxymethyl[AnyN-term] CarbamidomethylDTT[C] PhosphoHex[S] HexNAc[C] Ser->His[S] Biotin[AnyN-term] Cation_Cu[I][D] Met->Xle[M] Cytopiloyne[AnyN-term] Hex[S] Trp->Oxolactone[W] SPITC_13C(6)[AnyN-term] Xle->Asp[L] Cation_Fe[II][E] Deamidated_18O(1)[N](Delta_H(1)N(-1)18O(1)[N]) Cation_Fe[III][D] Nitrosyl[C] Nmethylmaleimide+water[C] SPITC[AnyN-term] Carboxyethyl[H](Ethoxyformyl[H]) Didehydro[T] Deoxy[S](Ser->Ala[S]) Asn->Thr[N] Thiazolidine[W] Hex(1)HexNAc(1)[S] Phosphogluconoylation[AnyN-term] Arg->Gln[R] 2-succinyl[C] AEC-MAEC[S] SulfanilicAcid_13C(6)[E] HMVK[C] Tyr->Ser[Y] dHex[S] Asn->Xle[N] PEITC[AnyN-term] Asp->Asn[D] ISD_z+2_ion[AnyN-term] Ala->Thr[A] ICPL_13C(6)2H(4)[AnyN-term] Unknown_216[AnyN-term] Cation_Ni[II][D] Nitro[Y] Formyl[S](Ser->Asp[S]) Cysteinyl[C] Carbofuran[S] Didehydro[S] Menadione-HQ[C] Sulfo[Y] Tyr->Phe[Y] Cation_Fe[II][D] NDA[AnyN-term] HexNAc(1)dHex(1)[T] Delta_H(6)C(3)O(1)[C] Thiophospho[S] Ser->Gly[S] Lys->Arg[K] Val->Pro[V] Formyl[T](Thr->Glu[T]) Cys->Gln[C] Phe->Trp[F] Arg[AnyN-term] Glutathione[C] Diisopropylphosphate[AnyN-term] Propionamide[C] Gly->His[G] Pro->Asn[P] Hex(1)HexNAc(1)[T] Ethylphosphate[AnyN-term] Didehydro[Y] monomethylphosphothione[C]

Children & Old People	16	Oxidation[T] Oxidation[V] cGMP+RMP-loss[C] Carbonyl[L] CAF[AnyN-term] Oxidation[F](Phe->Tyr[F]) Oxidation[L] Cys->Oxoalanine[C] Dioxidation[F] Carbamidomethyl[H] Malonyl[C] Carbamidomethyl[K](Gly[K]) Dioxidation[V] Carbonyl[V] Trp->Kynurenin[W] Dioxidation[M]
Old People & Young People	10	Val->Tyr[V] Val->Phe[V] Carbonyl[Q] Crotonaldehyde[C] SulfanilicAcid[E] Dioxidation[C] Val->Trp[V] Carbamyl[K] NIC[AnyN-term](ICPL[AnyN-term]) Pro->pyro-Glu[P]
Children	122	Xle->Glu[L] Galactosyl[AnyN-term] Asn->Met[N] Methyl[R] Fluoro[A] Xle->Val[L] Val->His[V] Xle->Gln[I] Ser->Val[S] Delta_S(-1)Se(1)[C] Phospho[T] HCysteinyl[C] Methyl_2H(3)[E] Val->Xle[V] Val->Gln[V] Quinone[Y] Met-loss+Acetyl[ProteinN-termM] Saligenin[H] Hex(1)HexA(1)[T] Thr->Tyr[T] Gln->Phe[Q] Xlink_BMOE[C] Ala->Ser[A] BITC[C] Propionyl_13C(3)[AnyN-term] Thr->Trp[T] Carboxy[W] HexN[N] Cation_Al[III][E] Carbonyl[A] Phospho[C] Acetyl_2H(3)[AnyN-term] IBTP[C] DNPS[C] Thr->His[T] Cys->SecNEM_2H(5)[C] Oxidation[S] Phe->Asp[F] Xlink_EGS[115][K] Hex(3)[S] Cation_Al[III][D] Gly->Ala[G] Xle->Tyr[I] Asn->Cys[N] Thr->Asp[T] lapachenole[C] Lys->MetOx[K] Trp->Hydroxykynurenin[W] Methylthio[AnyN-term] Xle->Gln[L] HexNAc(2)Sulf(1)[T] Cys->Ala[C] Gln->Arg[Q] Xle->Met[L] Propiophenone[C] Ala->Phe[A] Pro->Thr[P] Tyr->His[Y] Cation_Zn[II][E] CarboxymethylDTT[C] MTSL[C] Cys->His[C] Oxidation[H] Methylthio[N] IDEnT[C] Formylasparagine[H] Tyr->Asn[Y] Leu->MetOx[L] Hex(1)HexA(1)[S] phenylsulfonylethyl[C] FTC[S] NEMsulfurWater[C] Met->Thr[M] dHex(1)Hex(1)[T] DeStreak[C] trifluoro[L] ICAT-H_13C(6)[C] Pro->Ala[P] Unknown_216[E] O-Et-N-diMePhospho[S] Pro->Gly[P] Asp->Arg[D] Amidine[K] MercaptoEthanol[T] Fluoro[Y] Lys[AnyN-term] Gly->Val[G] Glucuronyl[S] Methyl[H] Trimethyl[K](Propyl[K]) Lys->Asp[K] IGBP[C] Pro->Xle[P] MercaptoEthanol[S] DNCB_hapten[C] Dicarbamidomethyl[AnyN-term] Quinone[W] HN3_mustard[C] Methyl[E] DTT[C] Ala->Asn[A] Val->Asn[V] Arg->His[R] Carboxy[D] Cation_Zn[II][D] Diethylphosphothione[C] Fluoro[W] Formyl[K] Ub-VME[C] Met->Phe[M] Gly->Phe[G] Pro->HAVA[P] Thrbiotinhydrazide[T] Hex(2)[T] Asp->His[D] Xle->His[L] Iodo[Y] Acetyl_2H(3)[T] Unknown_177[E] GG[K](Dicarbamidomethyl[K]) Carbamyl[M] Xlink_DST[132][K]
Young People	156	GIST-Quat_2H(3)[AnyN-term] Ethyl+Deamidated[Q] Deamidated_18O(1)[Q] Hex(1)NeuGc(1)[S] Xle->Gly[I] Dethiomethyl[M] HexN[T] Methyl[K] Argbiotinhydrazide[R] Delta_H(2)C(2)[AnyN-term] Sulfide[C] Unknown_302[AnyN-term] Nmethylmaleimide[C] PhosphoCytidine[S] NEM_2H(5)[C] dNIC[AnyN-term] Lys->Gln[K] NeuAc[S] Met->Gln[M] Pent(1)HexNAc(1)[T] AccQTag[AnyN-term] Dehydrated[Y] Hex(1)NeuGc(1)[T] Ser->Xle[S] PhosphoHex(2)[N] SMA[AnyN-term] Thr->Arg[T] NEMsulfur[C] Arg->Cys[R] Ub-amide[C] Thiazolidine[K] Xle->Gly[L] Gly->Pro[G] PhosphoHexNAc[S] Xle->Thr[L] Gly->Asp[G] HexNAc(2)[N] Thr->Val[T] Pentose[T] Xle->Asp[I] Hex[AnyN-term] Glucosylgalactosyl[K] Phenylisocyanate_2H(5)[AnyN-term] Dimethyl[AnyN-term](Ethyl[AnyN-term]) Nethylmaleimide+water[C] Arg->Lys[R] HexNAc(1)NeuAc(1)[T] pyrophospho[S] Hex(1)Pent(2)Me(1)[S] Ethanedithiol[S] Xle->Lys[L] Thiophospho[T] Bacillosamine[N] HexNAc(1)NeuAc(1)[S] Diethyl[AnyN-term] Deamidated[R] Fluorescein[C] Gln->Lys[Q] azole[C] GIST-Quat[AnyN-term] Dansyl[AnyN-term] Cation_Ag[D] PyridoxalPhosphate[K] Propionyl[AnyN-

		term] PEITC[C] TNBS[AnyN-term] Ethyl+Deamidated[N] BEMAD_ST_2H(6)[S] ICAT-H[C] HexNAc[S] Biotin_Thermo-21328[AnyN-term] 3sulfo[AnyN-term] Lipoyl[K] Met->Glu[M] NeuGc[S] Carboxy[K] Delta_H(4)C(2)O(-1)S(1)[S](Ser->Met[S]) Methylamine[S] PhosphoHexNAc[T] Delta_H(5)C(2)[P] Dibromo[Y] NO_SMX_SIMD[C] Lys->Allysine[K] Amidine[AnyN-term] Pro->His[P] Cation_Cu[I][E] Ala->Trp[A] GluGluGlu[E] Guanidinyl[K] Glucuronyl[T] Lys->Thr[K] Oxidation[K] Pent(2)[S] Gln->His[Q] Piperidine[AnyN-term] azole[S] CLIP_TRAQ_4[AnyN-term] Nitro[W] Methylthio[C] Dehydro[C] Thr->Pro[T] Hex(1)Pent(1)[T] Thiazolidine[Y] Carboxyethyl[K] dHex(1)Hex(2)[S] Thr->Gly[T] Xle->Trp[I] Hex(1)Pent(2)[S] Oxidation+NEM[C] HexNAc(2)Sulf(1)[S] Hydroxymethyl[N] QTGG[K] HexNAc(2)[T] NO_SMX_SEMD[C] Glu->His[E] Cys->Arg[C] Xlink_DTSSP[174][K] Asn->Phe[N] SulfoGMBS[C] methylol[Y] Lys->Asn[K] Iminobiotin[AnyN-term] Pro->Gln[P] Xle->Val[I] SulfurDioxide[C] BITC[AnyN-term] G-H1[R] Arg->Npo[R] FNEM[C] HexNAc(1)dHex(1)[S] Glu->Lys[E] Thr->Cys[T] Hex(1)HexNAc(1)Sulf(1)[S] Hex(1)HexNAc(1)Sulf(1)[T] Malonyl[K] Gly->Ser[G] ICPL_13C(6)[AnyN-term] Ser->Asn[S] Val->Lys[V] PhosphoHex(2)[S] Tyr->Trp[Y] Tyr->Thr[Y] Xle->Phe[L] CresylSaligeninPhosphate[K] HexNAc(1)dHex(1)[N] Dihydroxyimidazolidine[R] Methyl[S](Ser->Thr[S]) Asp->Xle[D] Asp->Trp[D] Ser->Pro[S] Sulfo-NHS-LC-LC-Biotin[AnyN-term] Hex(1)Pent(1)[S] Unknown_306[AnyN-term] Carbamyl[S] s-GlcNAc[T] Lys->Glu[K]
Old People	18	NBS[W] Dioxidation[E] Trioxidation[F] methylsulfonylethyl[C] Delta_O(4)[W] Dioxidation[L] Sulfo[S] Carbonyl[I] Val->Glu[V] Isopropylphospho[Y] Met->Asp[M] Thiazolidine[F] Oxidation[Q] Trioxidation[Y] Xle->Tyr[L] Dioxidation[I] Pro->Phe[P] Phospho[Y]

附表 2 老年人独有修饰中共有修饰所属蛋白

Appendix Table 2 proteins of common modifications in unique modifications of old people

UniProtKB ID	UniProtKB	Status	Protein names	Gene names	Organism	Length
P02671	FIBA_HUMAN	reviewed	Fibrinogen alpha chain [Cleaved into: Fibrinopeptide A; Fibrinogen alpha chain]	FGA	Homo sapiens (Human)	866
B7Z8Q2	B7Z8Q2_HUMAN	unreviewed	cDNA FLJ55606, highly similar to Alpha-2-HS-glycoprotein		Homo sapiens (Human)	433
A0A024R8G3	A0A024R8G3_HUMAN	unreviewed	Prostaglandin D2 synthase 21kDa (Brain), isoform CRA_a (Testis tissue sperm-binding protein Li 63n)	PTGDS hCG_1780827	Homo sapiens (Human)	190

Q59EG0	Q59EG0_HUMAN	unreviewed	Basement membrane-specific heparan sulfate proteoglycan core protein variant		Homo sapiens (Human)	2,331
B4DPP6	B4DPP6_HUMAN	unreviewed	cDNA FLJ54371, highly similar to Serum albumin		Homo sapiens (Human)	618
A0A5C2FTR4	A0A5C2FTR4_HUMAN	unreviewed	IGL c1349_light_IGKV3-20_IGKJ5 (IGL c368_light_IGKV3-20_IGKJ5)		Homo sapiens (Human)	109
A0A5C2GLT3	A0A5C2GLT3_HUMAN	unreviewed	IG c1042_light_IGKV2-28_IGKJ4		Homo sapiens (Human)	113
B4DN59	B4DN59_HUMAN	unreviewed	cDNA FLJ52702, highly similar to Homo sapiens CD44 antigen (homing function and Indian blood group system) (CD44), transcript variant 4, mRNA		Homo sapiens (Human)	329
B5MCZ9	B5MCZ9_HUMAN	unreviewed	Glutaminyl-peptide cyclotransferase	QPCT	Homo sapiens (Human)	284
B2R4M6	B2R4M6_HUMAN	unreviewed	Protein S100 (S100 calcium-binding protein)		Homo sapiens (Human)	114
S4R471	S4R471_HUMAN	unreviewed	Protein AMBP	AMBP	Homo sapiens (Human)	193

B2R888	B2R888_HUMAN	unreviewed	Monocyte differentiation antigen CD14 (Myeloid cell-specific leucine-rich glycoprotein)		Homo sapiens (Human)	375
Q06AH7	Q06AH7_HUMAN	unreviewed	Transferrin	TF	Homo sapiens (Human)	698
F8W6P5	F8W6P5_HUMAN	unreviewed	Hemoglobin subunit beta	HBB	Homo sapiens (Human)	90
B7ZKJ8	B7ZKJ8_HUMAN	unreviewed	ITIH4 protein (Inter-alpha-trypsin inhibitor heavy chain H4)	ITIH4	Homo sapiens (Human)	935
Q9BS19	Q9BS19_HUMAN	unreviewed	Epididymis secretory sperm binding protein (HPX protein) (Hemopexin)	HPX	Homo sapiens (Human)	254
P19652	A1AG2_HUMAN	reviewed	Alpha-1-acid glycoprotein 2, AGP 2 (Orosomucoid-2, OMD 2)	ORM2 AGP2	Homo sapiens (Human)	201
B7Z8Q4	B7Z8Q4_HUMAN	unreviewed	cDNA FLJ56652, highly similar to Hemopexin		Homo sapiens (Human)	142
B4DHP9	B4DHP9_HUMAN	unreviewed	cDNA FLJ58062, highly similar to Homo sapiens CD99 antigen-like 2 (CD99L2), transcript variant 1, mRNA		Homo sapiens (Human)	187

A0A0U1RQQ4	A0A0U1RQQ4_HUMAN	unreviewed	Endothelial protein C receptor	PROCR	Homo sapiens (Human)	213
V9HW68	V9HW68_HUMAN	unreviewed	Epididymis luminal protein 214	HEL-214	Homo sapiens (Human)	470
Q6GMX0	Q6GMX0_HUMAN	unreviewed	Uncharacterized protein		Homo sapiens (Human)	236
A6NL76	A6NL76_HUMAN	unreviewed	Actin, alpha skeletal muscle	ACTA1	Homo sapiens (Human)	254
G3V4B4	G3V4B4_HUMAN	unreviewed	Plasma serine protease inhibitor	SERPINA5	Homo sapiens (Human)	86
B2R4R0	B2R4R0_HUMAN	unreviewed	Histone H4	HIST1H4J HIST1H4H, HIST1H4L, hCG_1640981	Homo sapiens (Human)	103
B4DPR2	B4DPR2_HUMAN	unreviewed	cDNA FLJ50830, highly similar to Serum albumin		Homo sapiens (Human)	523
B3KTU0	B3KTU0_HUMAN	unreviewed	cDNA FLJ38724 fis, clone KIDNE2010151, highly similar to UROMODULIN		Homo sapiens (Human)	577

Q05CF8	Q05CF8_HUMAN	unreviewed	KNG1 protein	KNG1	Homo sapiens (Human)	291
B7ZMD7	B7ZMD7_HUMAN	unreviewed	Alpha-amylase, EC 3.2.1.1	AMY1A	Homo sapiens (Human)	511
J3QSF7	J3QSF7_HUMAN	unreviewed	Myeloperoxidase	MPO	Homo sapiens (Human)	86
P05109	S10A8_HUMAN	reviewed	Protein S100-A8 (Calgranulin-A) (Calprotectin L1L subunit) (Cystic fibrosis antigen, CFAG) (Leukocyte L1 complex light chain) (Migration inhibitory factor-related protein 8, MRP-8, p8) (S100 calcium-binding protein A8) (Urinary stone protein band A)	S100A8 CAGA, CFAG, MRP8	Homo sapiens (Human)	93
V9HWI4	V9HWI4_HUMAN	unreviewed	Epididymis luminal protein 110	HEL110	Homo sapiens (Human)	710
A0A0G2JRN3	A0A0G2JRN3_HUMAN	unreviewed	Alpha-1-antitrypsin	SERPINA1	Homo sapiens (Human)	359
A0A0U1RQK7	A0A0U1RQK7_HUMAN	unreviewed	Eukaryotic translation initiation factor 4 gamma 3	EIF4G3	Homo sapiens (Human)	1,774
H7C5N5	H7C5N5_HUMAN	unreviewed	Ceruloplasmin	CP	Homo sapiens (Human)	225

A0A024R1M3	A0A024R1M3_HUMAN	unreviewed	HGFL gene, isoform CRA_a	MGC17330 hCG_41521	Homo sapiens (Human)	263
B2RCB8	B2RCB8_HUMAN	unreviewed	cDNA, FLJ95971, highly similar to Homo sapiens protocadherin 12 (PCDH12), mRNA		Homo sapiens (Human)	1,184
D9ZGG2	D9ZGG2_HUMAN	unreviewed	Vitronectin	VTN	Homo sapiens (Human)	478
B4E1C2	B4E1C2_HUMAN	unreviewed	Kininogen 1, isoform CRA_b (cDNA FLJ56836, highly similar to Kininogen-1)	KNG1 hCG_2021397	Homo sapiens (Human)	644
A0A5C2GH36	A0A5C2GH36_HUMAN	unreviewed	IG c401_light_IGKV3-20_IGKJ4		Homo sapiens (Human)	107
H0Y5A1	H0Y5A1_HUMAN	unreviewed	Prostaglandin-H2 D-isomerase	PTGDS	Homo sapiens (Human)	124
E7EQR8	E7EQR8_HUMAN	unreviewed	Protein YIPF3	YIPF3	Homo sapiens (Human)	356
V9HWF6	V9HWF6_HUMAN	unreviewed	Alpha-1-acid glycoprotein	HEL-S-153w	Homo sapiens (Human)	201

W0UV60	W0UV60_HUMAN	unreviewed	Ribonuclease A F3 (Ribonuclease, RNase A family, 2 (Liver, eosinophil-derived neurotoxin))	RAF3 RNASE2, hCG_1778873	Homo sapiens (Human)	161
B7Z8R6	B7Z8R6_HUMAN	unreviewed	cDNA FLJ51445, highly similar to AMBP protein		Homo sapiens (Human)	270
D6CHE9	D6CHE9_HUMAN	unreviewed	Proteinase 3 (Serine proteinase, neutrophil, Wegener granulomatosis autoantigen), isoform CRA_a	PRTN3 hCG_1811455	Homo sapiens (Human)	215
H3BNC6	H3BNC6_HUMAN	unreviewed	Cadherin-1	CDH1	Homo sapiens (Human)	537
C9J2Z5	C9J2Z5_HUMAN	unreviewed	Alpha-amylase 2B	AMY2B	Homo sapiens (Human)	96
Q9BV73	CP250_HUMAN	reviewed	Centrosome-associated protein CEP250 (250 kDa centrosomal protein, Cep250) (Centrosomal Nek2-associated protein 1, C-Nap1) (Centrosomal protein 2)	CEP250 CEP2, CNAP1	Homo sapiens (Human)	2,442